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Patentanmeldung Nr. / Patent application No. / Demande de brevet n°

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Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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Axxima Pharmaceuticals AG
Max-Lebsche-Platz 32
81377 München
ALLEMAGNE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective compounds against infectious diseases

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4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective compounds against infectious diseases.

Description

The present invention relates to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues and pharmaceutically acceptable salts thereof, the use of these derivatives for the prophylaxis and/or treatment of mycobacteria-induced infections and opportunistic infections, as well as compositions containing at least one 4,7-dihydro-5H-thieno[2,3c]pyran derivative or analogue thereof and/or pharmaceutically acceptable salts thereof.

Mycobacteria is the cause for a number of severe diseases, among them tuberculosis, leprosy, and mycobacteria-induced meningitis. Tuberculosis is an ancient scourge of human beings, caused by *Mycobacterium tuberculosis*. Although more than three billion people have been inoculated with the vaccine BCG, presently more than 50,000 people die every week of tuberculosis world-wide, and there are estimations that one third of the world's population is infected by *Mycobacterium tuberculosis*. According to a recent report of the World Health Organisation (WHO) on tuberculosis epidemic, distributed via the internet, it is estimated that between the years 2000 and 2020, nearly one billion people will carry tuberculosis bacteria, 200 million people will get sick, and 35 million will die of tuberculosis, if control of the disease and preventive measures are not strengthened. Moreover, it has been reported that 32% of HIV infected individuals die of tuberculosis. The situation has become even more dramatic since a number of *Mycobacterium tuberculosis* strains have shown a multidrug resistance, which cannot be attacked by conventional therapy, e.g. antibiotics. In addition, immune-suppressed people like AIDS patients are often victims of mycobacterial infections leading to a poor prognosis.

There are several reasons why mycobacteria-induced diseases are difficult to cure: First of all, mycobacteria can perform a differentiation process called "dormancy" or "persistency". Dormant mycobacteria are much more resistant against conventional antibacterial drug treatment. Secondly, many of the mycobacteria species have long replication times, resulting in a slow growth. One consequence thereof is that antimycobacterial drugs need longer medication times compared to the medication of

faster growing pathogenic bacteria. Both factors cited above are reasons why a medical treatment of mycobacteria-induced diseases has to last at least for several months. A third factor why conventional antibacterial drug treatment is so difficult with regard to mycobacteria-induced diseases is that these bacteria have a relatively thick cell wall, which is not or almost not permeable for many substances.

The use of 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives in the treatment of mycobacterial infections such as tuberculosis are described in the as yet unpublished PCT patent application PCT/EP03/03697. The compounds described therein have been found to be effective in blocking the activity of mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), which have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacterial within a macrophage cell line, and thereby provide a mode for the elimination of mycobacteria.

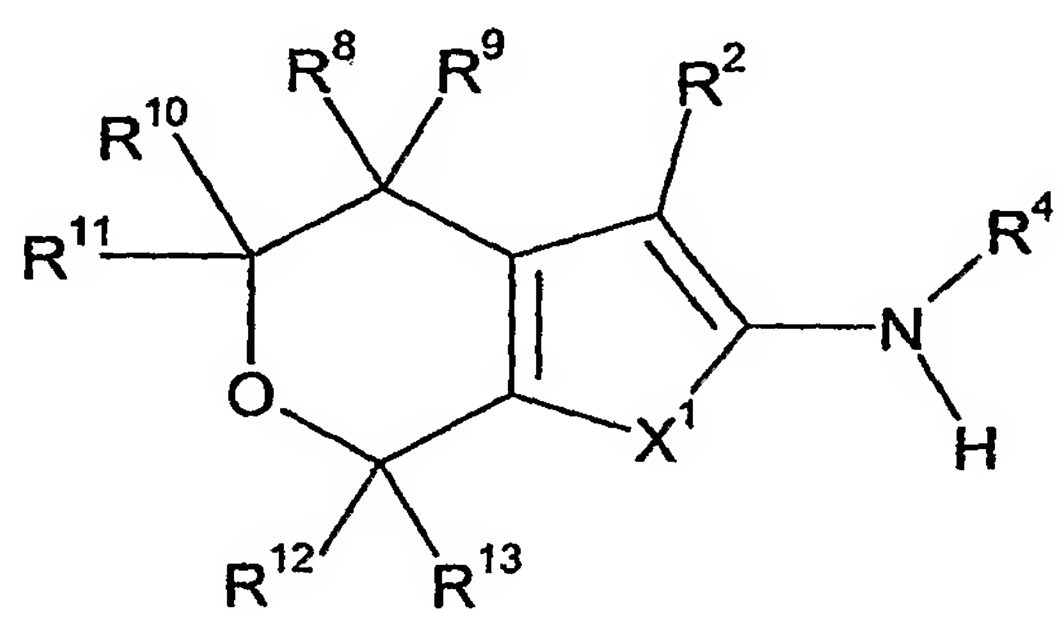
Additionally, biologically active 4,7-dihydro-5-H-thieno[2,3c]pyran and 4,7-dihydro-5-H-thieno[2,3-c]thiopyran derivatives are described in *Biorg. Med. Chem. Letters* **2002**, *12*, 1897-1900, in which compounds which inhibit TNF-a-production are described, in *J. Med. Chem.* **2002**, *45*, 4443-4459, in which compounds are described which act as protein-tyrosine phosphatase 1B (PTP1B) inhibitors, or in Japanese patent JP 2002308870, in which compounds are described, which act as Staphylococcus aureus inhibitors. Further derivatives are described in *Armyanskii Khimicheskii Zhurnal* **1987**, 40(9), 581-7. These references do not disclose any PkNG inhibitory activity for these compounds.
In WO 01/98290 thiophene derivatives are described as active kinase inhibitors.

One important feature for pharmaceutical active agents in general is that these agents have a high degree of metabolitic stability. It was found that the compounds described in PCT/EP03/03697, while being pharmaceutically active as PkNG inhibitors, left room for further increase of metabolitic stability.

Taking into account the above-mentioned problems with conventional antimycobacterial treatment, it is the object of the present invention to provide compounds and/or pharmaceutically acceptable salts thereof which can be used as pharmaceutically active substances, especially for the prophylaxis and/or treatment of mycobacteria-induced infections, a method to treat mycobacteria-induced diseases by means of those compounds, as well as compositions comprising at least one of those compounds and/or pharmaceutically acceptable salts thereof as pharmaceutically active ingredients.

This object is solved by the 4,7-Dihydro-5H-thieno[2,3c]pyran derivative and analogous compounds and/or their pharmaceutically acceptable salts of independent claim 1, the use of at least one of the those compounds and/or the pharmaceutically acceptable salts thereof as pharmaceutically active agents according to independent claims 24 or 35, the use of the compounds as an inhibitor for a protein kinase according to independent claims 30, the use of at least one compound and/or a pharmaceutically active salt thereof for the preparation of a pharmaceutical composition according to independent claim 46, the method for preventing of treating a disease or infection in a mammal according to independent claim 55, and a method of amidation of a carboxylic acid ester according to independent claim 60. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, the examples and the drawings.

The 4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof according to the present invention are represented by the following general formula (I)



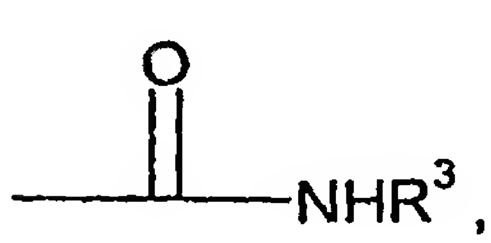
(I)

wherein

X^1 is selected from S, O, NR^1,

and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is selected from



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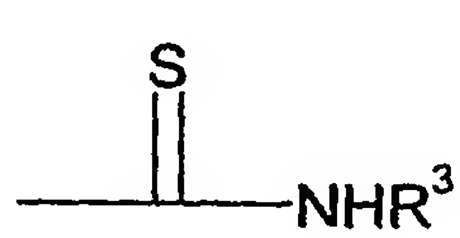

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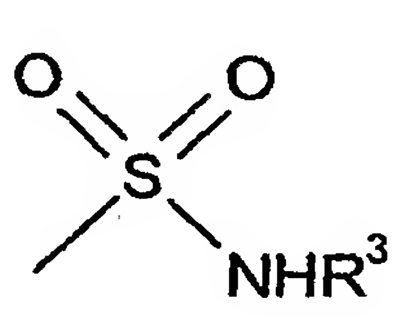

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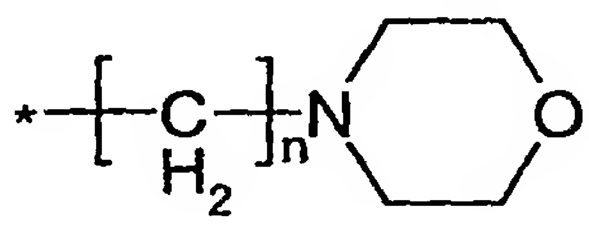
wherein R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_6-alkyl,

R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,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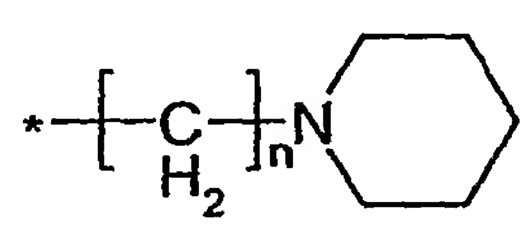
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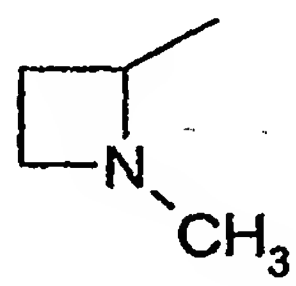
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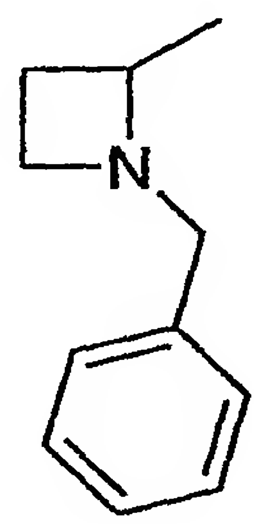
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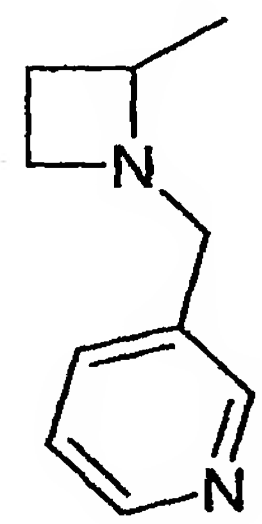
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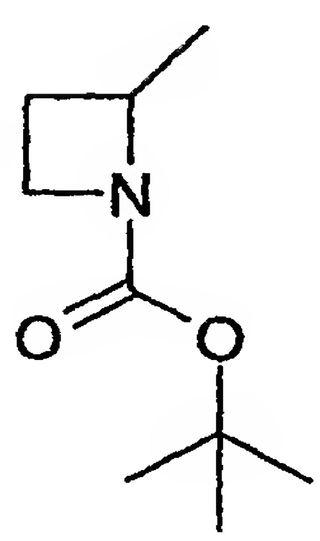
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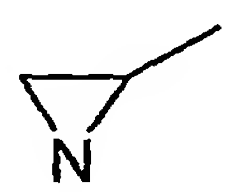
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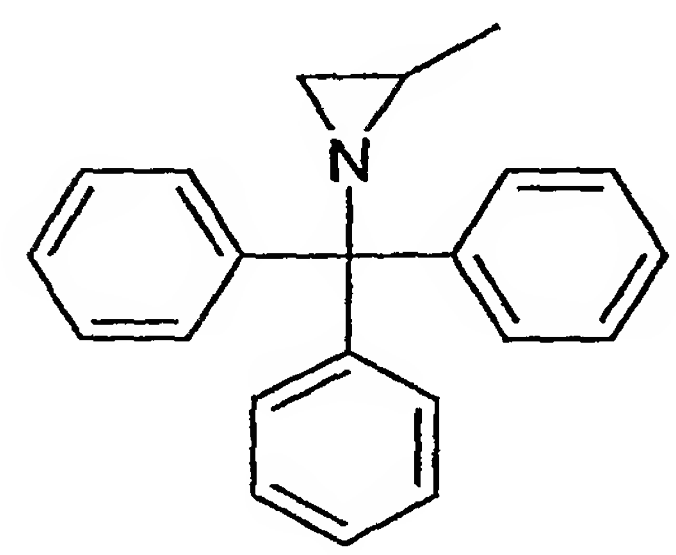
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or -$(CH_2)_n$-$NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and

R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

and include stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

As used in the present invention, the term substituted or unsubstituted C_1-C_6-alkyl or C_1-C_4-alkyl or C_1-C_3-alkyl is meant to include linear or branched alkyls in which optionally one, two or three of the hydrogen atoms bonded to the carbon chain are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl, a -OH or -SH group, a $-NH_2$ group, methoxy or ethoxy group, or phenyl group. These terms therefore especially comprise, depending on the number of carbon atoms in each respective term, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert.-butyl, $-C_5H_{11}$, $-CH_2-C(CH_3)_3$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, $-CH_2-CH(CH_3)-C_3H_7$, $-CH(CH_3)-CH_2-CH(CH_3)_2$, $-CH(CH_3)-CH(CH_3)-C_2H_5$, $-CH_2-CH(CH_3)-CH(CH_3)_2$, $-CH_2-C(CH_3)_2-C_2H_5$, $-C(CH_3)_2-C_3H_7$, $-C(CH_3)_2-CH(CH_3)_2$, $-C_2H_4-C(CH_3)_3$, $-CH(CH_3)-C(CH_3)_3$, optionally substituted in the above described manner, especially to give phenyl substituted alkyles such as benzyl.

Similarly, the term substituted or unsubstituted C_3-C_6-cycloalkyl is meant to include cyclolalkanes in which optionally one, two or three of the hydrogen atoms bonded to the carbon atoms of the cycle are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl, a -OH or -SH group, a $-NH_2$, methoxy or ethoxy or methyl, ethyl or phenyl group. This term therefore includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl as well as methyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, ethyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or phenyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, optionally substituted in the above described manner.

Similarly, the term unsubstituted or substituted C_2-C_4-alkenyl is meant to include branched or linear alkenyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl. These terms therefore are meant to comprise ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl , but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, optionally substituted in the above described manner.

Similarly, the term unsubstituted or substituted C_2-C_4-alkinyl is meant to include branced or linear alkinyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl. These terms therefore are meant to comprise prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, and but-3-inyl, optionally substituted in the described above manner.

The term substituted or unsubstituted aryl is meant to include aromatic compounds, in which one, two or three of the hydrogen atoms bonded to the aromatic ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by $-NO_2$, .OH, -SH, $-NH_2$, -CN, methyl or methoxy. This term is therefore meant to comprise phenyl, 2,3-halogen substituted phenyl, 3,4-halogen substituted phenyl.

The term substituted or unsubstituted heteroaryl is meant to include aromatic groups in which the aromatic ring comprises at least one heteroatom selected from the group N, O, or S, and in which one, two or three of the hydrogen atoms bonded to the aromatic ring are optionally substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by $-NO_2$, -OH, -SH, methyl or methoxy. This term therefore includes furanyl, pyrollyl, thienyl, and pyridinyl which optionally can be substituted in the above described manner.

The term substituted or unsubstituted heterocycloalkyl is meant to include cycloalkyles in which at least one of the carbon atoms of the ring, preferably 1 or 2 atoms, have been substituted by a heteroatom selected from the group consisting of N, O, and S which optionally and in which one, two or three of the hydrogen atoms bonded to the ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by methyl or methoxy. This term therefore includes pyrrolidinyl, piperidinyl and tetrahydrofuranyl, which optionally can be substituted in the above described manner.

In a preferred embodiment of the present invention X^1 is S.

In a further preferred embodiment of the present invention X^1 is NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

In a further preferred embodiment of the present invention X^1 is O.

In a further preferred embodiment of the present invention R^2 is $-C(=O)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In a further preferred embodiment of the present invention R^2 is $-C(=S)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In a further preferred embodiment of the present invention R^2 is -SO_2NHR^3 and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In yet another preferred embodiment of the invention R^3 is selected from the group consisting of H, -CH_2-CH_2-OH, -CH_2-CH_2-NH_2, -CH_2-CH_2-SH, -CH_2-CH(OH)-CH_3, -CH_2-CH(SH)-CH_3, or -CH_2-CH(NH_2)-CH_3.

In a further preferred embodiment of the present invention R^4 is -C(=X^2)R^5 and X^2 is O or S, and preferably O.

In a further preferred embodiment of the present invention R^4 is -SO_2-R^5.

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl , but-1-enyl, but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, adamantyl, or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl , but-1-enyl, but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, or adamantyl.

In yet another preferred embodiment of the present invention R_5 is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl, prop-1-enyl, but-1-enyl, adamantyl, 3,4-difluorophenyl or NR^6R^7, wherein R^6 is H and R^7 is R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^7 preferably is phenyl or 3,4-difluorophenyl.

In another preferred embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl. In a further embodiment of the present invention, the compound 5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide is excluded from the compounds according to the present invention.

In yet another embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkoxy, or OH.

In yet another preferred embodiment of the present invention R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1-C_6-alkyl.

In a further preferred embodiment of the present invention R^8 and R^9 are both H.

In a further preferred embodiment of the present invention R^{10}, R^{11}, R^{12}, and R^{13} are independently selected from H and substituted or unsubstituted C_1-C_6-alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl or tert.-butyl.

In yet another preferred embodiment of the present invention R^{10} and R^{11} are methyl and R^{12} and R^{13} are H, or R^{10}, R^{11}, R^{12}, and R^{13} are H, or R^{10}, R^{11}, R^{12}, and R^{13} are methyl, or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.

In yet another preferred embodiment of the present invention R^{10} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

In yet another preferred embodiment of the present invention R^{12} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

In a further preferred embodiment of the present invention R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl or benzyl.

In a further preferred embodiment of the present invention R_{14} and R_{15} are independently selected from methyl, ethyl and propyl or allyl, and preferably are methyl.

In yet another preferred embodiment of the invention compound according to formula (I) is selected from the group consisting of:

(Compound 1) 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 2) 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 3) 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 4) 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 5) 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 6) 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 7) 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 8) 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 9) 2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 10) 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 11) 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 12) 2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 13) 2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 14) 2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 15) 2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 16) 5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, and

(Compound 17) 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide

The present invention also comprises pharmaceutically active salts of these compounds, all stereoisomeric forms and regioisomeric forms of these compounds or prodrugs thereof.

Other aspects of the present invention relate to the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as outlined above in the general formula (I) for use as new pharmaceutically active agents, particularly for the prophylaxis and/or treatment of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or mycobateria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy, pharmaceutical compositions comprising these 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as active ingredients and a method for treating virally and/or bacterially induced diseases, particularly mycobacteria-induced infections, in mammals, including humans, especially for the treatment of treatment of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or mycobateria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy.

Surprisingly, it was found that the compounds according to the present invention as well as pharmaceutically acceptable salts of these derivatives are effective against virally and/or bacterially induced diseases, especially mycobacteria-induced infections and diseases at pharmaceutically acceptable concentrations while exhibiting enhanced metabolitic stability.

Additionally, the present invention relates to the use of the compounds of the present invention for the manufacturing of a pharmaceutical composition for the prophylaxis and/or treatment of virally and/or bacterially induced diseases, particularly those infections and diseases mentioned above.

The compounds of the present invention are effective against mycobacteria induced infections, particularly tuberculosis, but also e.g. leprosy and mycobacteria-induced meningitis. Mycobacteria which induce or cause these infectious diseases are members of the group comprising the tuberculous bacteria *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum* and *M. leprae* as well as the non-tuberculous bacteria *M. abscessus*, *M. avium*, *M. celatum*, *M. chelonae*, *M. fortuitum*, *M.*

genavense, *M. gordonae*, *M. haemophilum*, *M. intracellulare*, *M. kansii*, *M. malmoense*, *M. marinum*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans* and *M. xenopi*. Because of the outstanding clinical importance of tuberculosis, microbiologists have distinguished the so-called "Mycobacterium tuberculosis complex" consisting of *Mycobacterium tuberculosis*, *M. bovis*, and *M. africanum* from all other mycobacteria which form the group of the so-called "atypical mycobacteria" or "non-tuberculous mycobacteria (NTM)".

The present invention also provides a method for preventing or treating infections and diseases, especially virally or bacterially induced diseases or infections, more specially infections induced by bacteria of the genus legionella such as legionaires disease, mycobacteria-induced infections (including opportunistic infections) in mammals (including humans), which method comprises administering to the mammal an pharmaceutically effective amount of the compounds of the present invention to treat a infection or disease. Especially, the method is used for the treatment of tuberculosis, but also for other mycobacteria-induced infections like leprosy or mycobacteria-induced meningitis.

According to a still further aspect, the present invention refers to pharmaceutical compositions comprising at least one compound according to the present invention as an active ingredient together with at least one pharmaceutically acceptable (i.e. non-toxic) carrier, excipient and/or diluent. The pharmaceutical compositions of the present invention can be prepared in a conventional solid or liquid carrier or diluent and a conventional pharmaceutically-made adjuvant at suitable dosage level in a known way. The preferred preparations are adapted for oral application. These administration forms include, for example, pills, tablets, film tablets, coated tablets, capsules, powders and deposits.

Furthermore, the present invention also includes pharmaceutical preparations for parenteral application, including dermal, intradermal, intragastral, intracutan, intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical, or transdermal application, which preparations in addition to typical vehicles and/or diluents contain at least one compound according to the present invention and/or a pharmaceutical acceptable salt thereof as active ingredient.

The pharmaceutical compositions according to the present invention containing at least one compound according to the present invention, i.e. one 4,7-Dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof as set out in general

formula (I) in independent claim 1 or claims dependent thereon, and/or a pharmaceutical acceptable salt thereof as active ingredient will typically be administered together with suitable carrier materials selected with respect to the intended form of administration, i.e. for oral administration in the form of tablets, capsules (either solid filled, semi-solid filled or liquid filled), powders for constitution, gels, elixirs, dispersable granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable carrier, preferably with an inert carrier like lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid filled capsules) and the like. Moreover, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated into the tablet or capsule. Powders and tablets may contain about 5 to about 95 weight % of the 4,7-dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof or the respective pharmaceutically active salt as active ingredient.

Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among suitable lubricants there may be mentioned boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Suitable disintegrants include starch, methylcellulose, guar gum, and the like. Sweetening and flavoring agents as well as preservatives may also be included, where appropriate. The disintegrants, diluents, lubricants, binders etc. are discussed in more detail below.

Moreover, the pharmaceutical compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimise the therapeutic effect(s), e.g. antihistaminic activity and the like. Suitable dosage forms for sustained release include tablets having layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices.

Liquid form preparations include solutions, suspensions, and emulsions. As an example, there may be mentioned water or water/propylene glycol solutions for parenteral injections or addition of sweeteners and opacifiers for oral solutions,

suspensions, and emulsions. Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be present in combination with a pharmaceutically acceptable carrier such as an inert, compressed gas, e.g. nitrogen.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides like cocoa butter is melted first, and the active ingredient is then dispersed homogeneously therein e.g. by stirring. The molten, homogeneous mixture is then poured into conveniently sized moulds, allowed to cool, and thereby solidified.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions, and emulsions.

The compounds according to the present invention may also be delivered transdermally. The transdermal compositions may have the form of a cream, a lotion, an aerosol and/or an emulsion and may be included in a transdermal patch of the matrix or reservoir type as is known in the art for this purpose.

The term capsule as recited herein refers to a specific container or enclosure made e.g. of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredient(s). Capsules with hard shells are typically made of blended of relatively high gel strength gelatins from bones or pork skin. The capsule itself may contain small amounts of dyes, opaquing agents, plasticisers and/or preservatives.

Under tablet a compressed or moulded solid dosage form is understood which comprises the active ingredients with suitable diluents. The tablet may be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation, or by compaction well known to a person of ordinary skill in the art.

Oral gels refer to the active ingredients dispersed or solubilised in a hydrophilic semi-solid matrix.

Powders for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended e.g. in water or in juice.

Suitable diluents are substances that usually make up the major portion of the composition or dosage form. Suitable diluents include sugars such as lactose, sucrose, mannitol, and sorbitol, starches derived from wheat, corn rice, and potato, and celluloses such as microcrystalline cellulose. The amount of diluent in the composition can range from about 5 to about 95 % by weight of the total composition, preferably from about 25 to about 75 weight %, and more preferably from about 30 to about 60 weight %.

The term disintegrants refers to materials added to the composition to support break apart (disintegrate) and release the pharmaceutically active ingredients of a medicament. Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar, cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses, and cross-linked microcrystalline celluloses such as sodium croscaramellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures. The amount of disintegrant in the composition may range from about 2 to about 20 weight % of the composition, more preferably from about 5 to about 10 weight %.

Binders are substances which bind or "glue" together powder particles and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation. Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include sugars such as sucrose, starches derived from wheat corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulose materials such as methylcellulose, sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganic compounds such as magnesium aluminum silicate. The amount of binder in the composition may range from about 2 to about 20 weight % of the composition, preferably from about 3 to about 10 weight %, and more preferably from about 3 to about 6 weight %.

Lubricants refer to a class of substances which are added to the dosage form to enable the tablet granules etc. after being compressed to release from the mould or die by reducing friction or wear. Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate, or potassium stearate, stearic acid, high melting point waxes, and other water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and D,L-leucine. Lubricants are usually added at the very last step before compression, since

they must be present at the surface of the granules. The amount of lubricant in the composition may range from about 0.2 to about 5 weight % of the composition, preferably from about 0.5 to about 2 weight %, and more preferably from about 0.3 to about 1.5 weight % of the composition.

Glidents are materials that prevent caking of the components of the pharmaceutical composition and improve the flow characteristics of granulate so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition may range from about 0.1 to about 5 weight % of the final composition, preferably from about 0.5 to about 2 weight %.

Coloring agents are excipients that provide coloration to the composition or the dosage form. Such excipients can include food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide. The amount of the coloring agent may vary from about 0.1 to about 5 weight % of the composition, preferably from about 0.1 to about 1 weight %.

To identify substances for drug development against mycobacteria-induced diseases, it was searched for inhibitors of signal transduction components present in mycobacteria. As already mentioned above, the elimination of mycobacteria from the human body is presently achieved by inhibiting the growth of respective bacteria by means of antibiotics. According to the present invention, a novel strategy has been used to fight against mycobacteria, namely to attack mycobacterial signal transduction components which are involved in the persistence of the bacteria within the host cell. Previously, it had been shown that mycobacteria penetrate cells via the endocytotic pathway. Endosomes containing non-pathogenic mycobacteria fuse to lysosomes and subsequently the bacteria are degraded by lysosomal enzymes. However, pathogenic mycobacteria, like *Mycobacterium tuberculosis*, contain additional "virulence genes" which prevent fusion of endosomes and lysosomes and thus circumvent the degradation within a host cell.

Mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacteria within a macrophage cell line. Furthermore, it could be demonstrated that the activity of PknG is an essential factor for virulence of mycobacteria. In accordance with the present invention, compounds have been found which are blocking the activity of PknG in a submicromolar range thus showing that PknG is a suitable target for recognising diseases, monitoring diseases, and controlling therapy of diseases related to mycobacterial infections.

These compounds (inhibitors) were able to induce efficient degradation of mycobacteria within host cells so that the present invention provides a novel mode for elimination of mycobacteria.

It has been found that certain disease inducing factors can be secreted by a cellular organism to the environment of the organism. Specifically, in the present case it has been found that mycobacterial proteins are secreted from the bacterium *Mycobacterium tuberculosis* to the environment of such a bacterium. A protein, which can be secreted by *Mycobacterium tuberculosis* is the protein serine/threonine kinase PknG. The fact that the above-mentioned inventive compounds are particularly effective against PknG may be due to the fact that this protein kinase can be attacked by these compounds without the need to penetrate the (thick) cell wall of *Mycobacterium tuberculosis*.

The compounds according to the present invention are obtainable by different synthetic routes. One route, which leads to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives starts with the reaction of tetrahydro-pyran-4-one or a correspondingly substituted derivative thereof with an cyano-actetate ester under acidic or basic conditions, preferably under acidic conditions, and under elimination of water and subsequent reaction of the reaction product with sulfur in the presence of an organic base to give a corresponding 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative.

As a next step, the amino group in the thus obtained 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be acylated to give a corresponding 2-carbonylamino compound. As an acylation reagent a carboxylic acid chloride is preferably used. This reaction can optionally be carried out in the presence of a base such as an tertiary amide, preferably $NEt(^iPr)_2$.

Other suitable reactions to obtain the secondary carboxylic acid amides can be used, for instance reaction of the amino group with a carboxylic acid and a coupling-agent as used in peptide chemistry, such as HOBT,HOOBT,HBTU or HOAt.

Alternatively, if instead of the acyl group a sulfonyl group is to be attached to the amino group in 2-position, the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be reacted with a sulfonyl chloride compound to give a corresponding 2-sulfanylamino derivative.

The thus obtained compounds can then optionally be reacted with bromine in the presence of an organic acid, preferably acetic acid, to substitute one hydrogen in 7-position of the heterocyclic nucleus by a hydroxyl group.

The above described 3-carboxylic acid ester derivative compounds can then be reacted in a subsequent reaction step with an alkali metal amide, such as $LiNH_2$ or

$NaNH_2$, in a polar solvent, which is essentially inert to the alkali metal amide, to give the corresponding 3-carboxylic acid amide derivative. This reaction is preferably carried out under the exclusion of moisture and optionally under an inert atmosphere. The application of lithium amide instead of sodium amide results in higher yields and purer products

To prepare the corresponding 4,7-dihydro-5H-thieno[2,3-c]pyran derivatives in which a sulfonamide is attached in 3-position, in a first step, 4,7-dihydro-5*H*-thieno[2,3-c]pyran-2-amine can be acylated, preferably using a carboxylic acid chloride to give the corresponding 2-carbonyl-amino derivative. This compound can then be reacted with sulfurylchloride, preferably under an inert atmosphere and subsequently with ammonia to give the 3-sulfonamide compound.

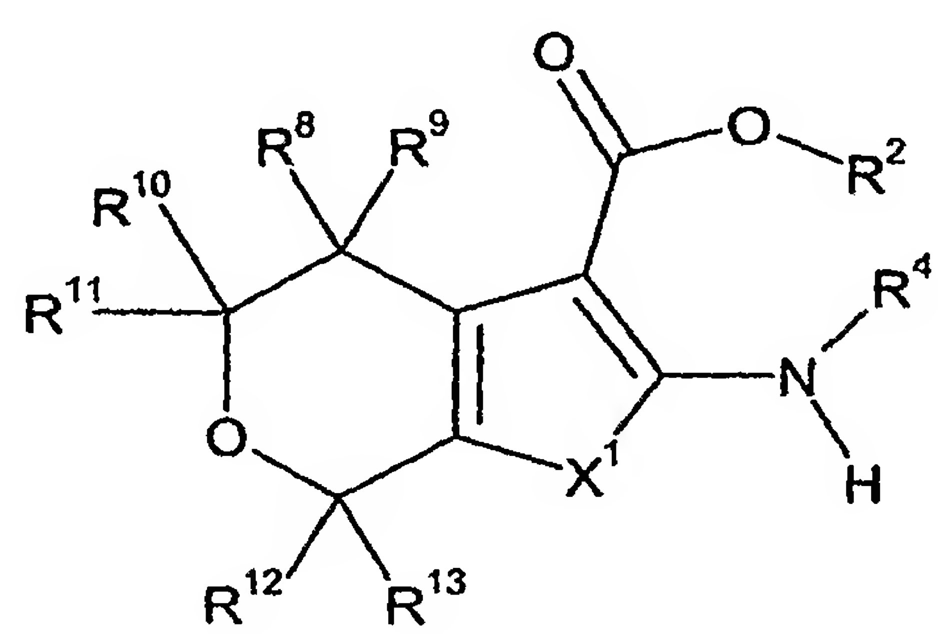
If the compounds used to synthesise the compounds according to the present invention contain -NH, -SH or -OH functional groups which potentially interfere with the desired reaction, these may of course be protected with suitable protective groups, which can later on be removed from the respective compounds.

To obtain those analogues of the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives in which the S-atom in the 5-membered ring of the heterocyclic nucleus is substituted either by NR^1 or O, the following synthetic approach can be utilized, which is partially based on a method described in Hauser, C.R., Hoffenberg, D.S.; *J.Org.Chem.* **1955**, *20*, 1448 - 1453.
To obtain the O-analogue compounds the amino group in 2-position a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane derivatives can be acylated in a first reaction step, using the acylation reaction described above with reference to the acylation of the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivatives, i.e. preferably using a carboxylic acid chloride as an acylation agent, obtionally in the presence of a tertiary amine base such as $NEt(^iPr)_2$.
Similarly, to obtain the NR^1-analogue compounds, a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane derivative is acylated in the above described manner.
The respective 2-carbonyl-amino derivatives obtained by this acylation can then be reacted with boron triflouride-acetic acid complex $[BF_3\cdot(HOAc)_2]$ and subsequently treated with an aqueous alkali metal hydroxide solution, such as sodium hydroxide, to convert the cyano group in 3-position of the heterocyclic nucleus into the carboxamide group.

In a further aspect of the present invention, the invention is directed at a method for amidation of an carboxylic acid ester to give the corresponding primary carboxylic acid amide. This amidation comprises the step of reacting an carboxylic acid ester with an alkali metal amide in the presence of a polar solvent, which is essentially inert against the alkali metal amide. Preferably, the molar ratio of carboxylic acid ester to alkali metal amide lies in the range of 1:1 to 1: 15., more preferably in the range of 1:5 to 1:13 and most preferably in the range of 1:9 to 1: 13.

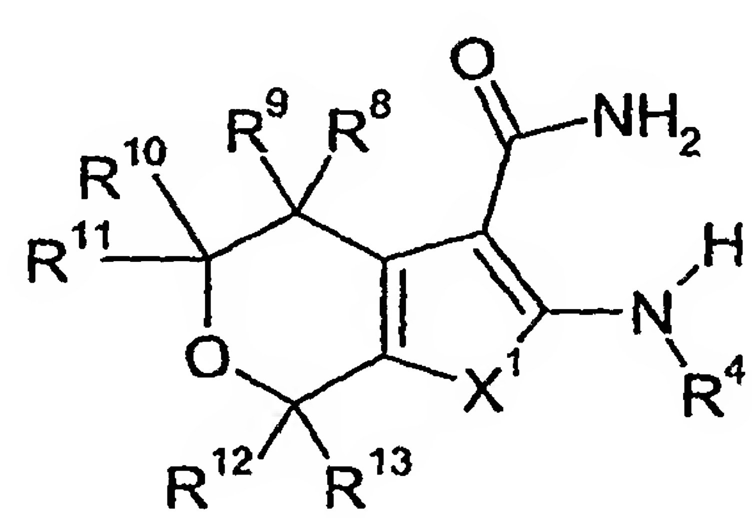
In a preferred embodiment of the method of the present invention, the alkali metal amide is $LiNH_2$ or $NaNH_2$, and preferably is $LiNH_2$. The solvent is preferably absolute ether or absolute tetrahydrofurane, preferably tetrahydrofurane, and the reaction is preferably carried out under the exclusion of moisture. Preferably, the reaction is carried out at a temperature of 15°C to 35°C, preferably at 25°C. It is furthermore preferred that the reaction duration lies in the range of from 40 to 80 hours, preferably from 45 to 75 hours.

In a preferred embodiment of the method of the present invention, the carboxylic acid ester is a compound according to the following general formula (D):



(D)

which is amidated to give the primary carboxylic acid amide according to formula (E),



(E)

wherein in formulas (D) and (E)

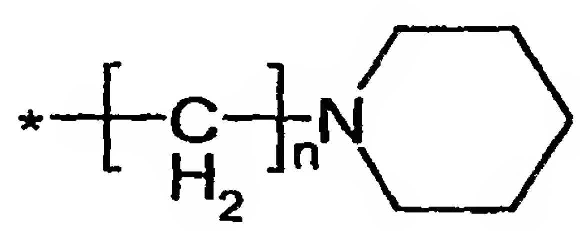
X^1 is selected from S, O, or NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is linear or branched C_1-C_6 alkyl or aryl and preferably is methyl, ethyl, phenyl or benzyl,

R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,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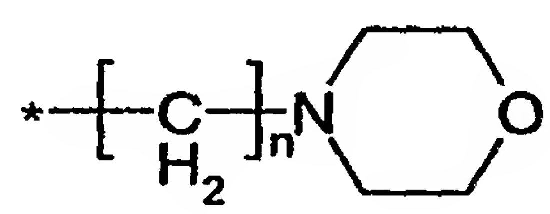
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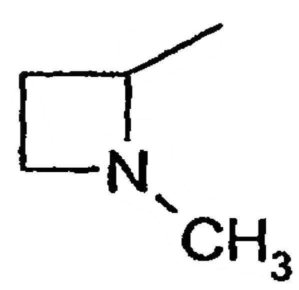
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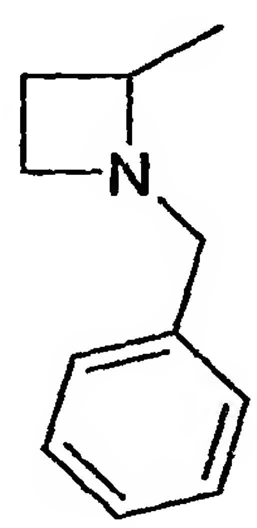
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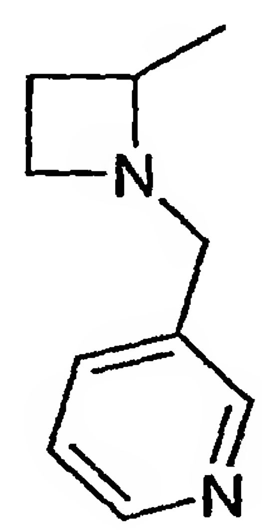
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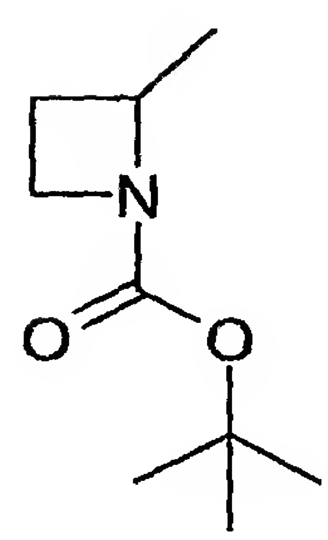
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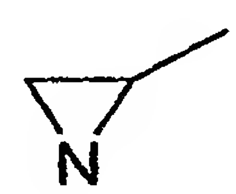
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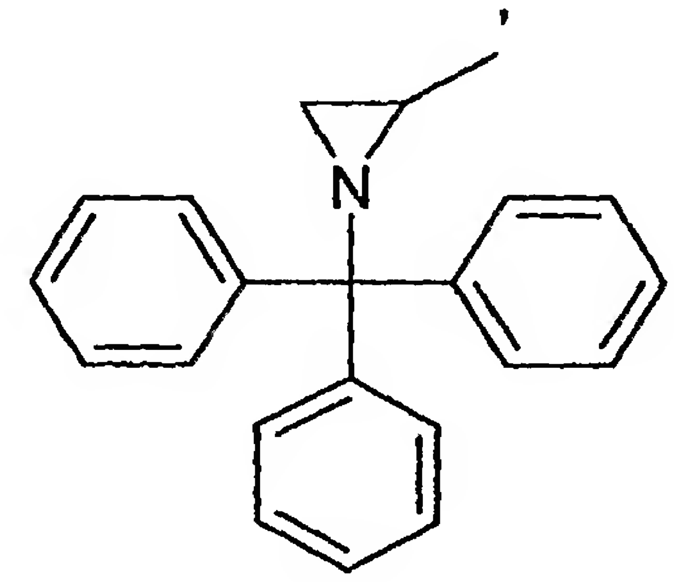

,

or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and

R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

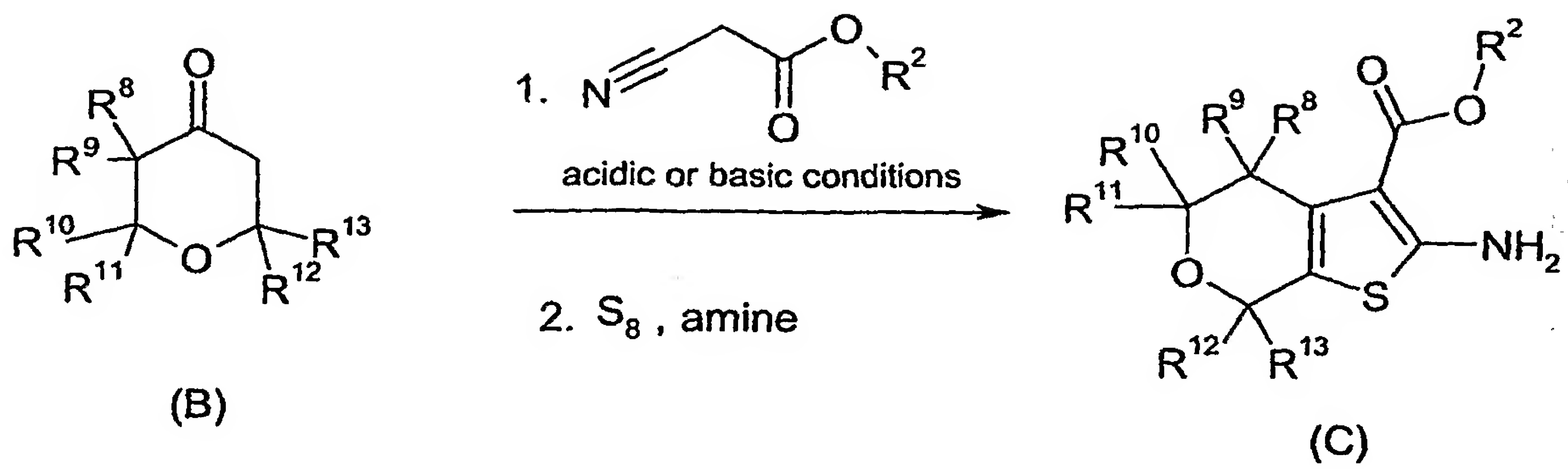
R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl
R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl
R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and
R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

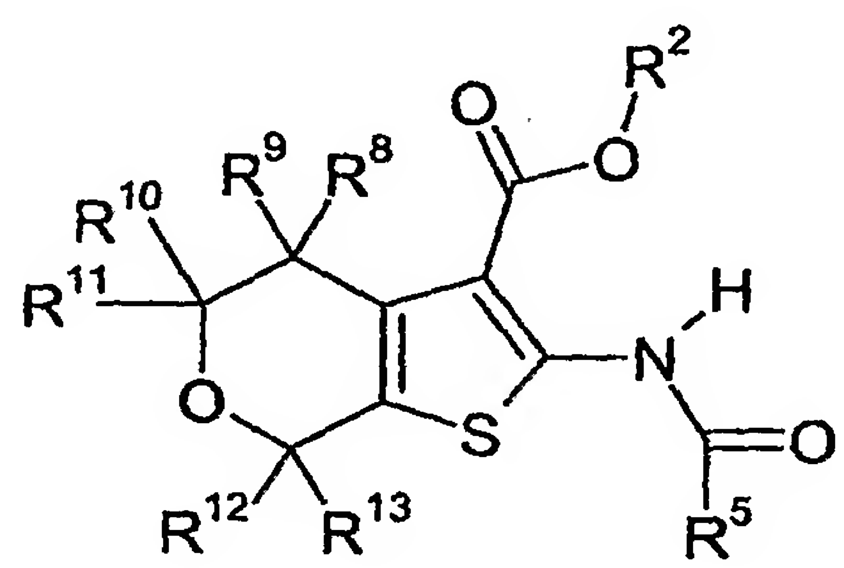
In a further preferred embodiment of the method of the present invention, in general formulas (D) and (E)
X^1 is S
R^2 is methyl or ethyl,
R^4 is -C(=O)R^5 and R5 is selected from methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl or adamantyl,
R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,
R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH,
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl,
R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and
R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

According to one preferred embodiment of the method of the present invention, the compound according to the general formula (E) is obtained by the following reaction sequence:

Step I:



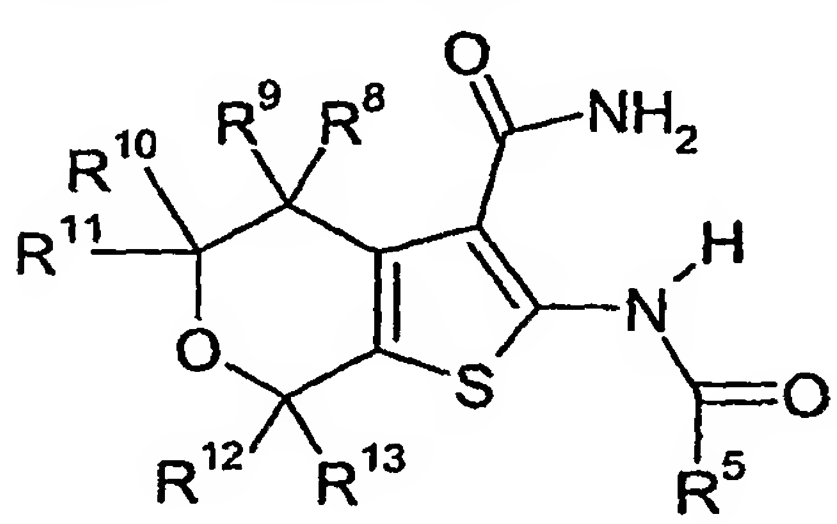
Step II: acylation of the -NH_2 group in 2-position in compound C with $R^5C(=O)LG$, wherein LG represents a suitable leaving group, preferably a halogen such as F, Cl, Br or I, most preferably Cl, to give compound (D):



(D)

and,

Step III: Amidation of as outlined in any one of claims 60 to 65, to give compound (E):



(E)

It is preferred that in Step I the reaction of compound (B) with the cyano-acetate ester is carried out in a nonpolar solvent, preferably benzene, with the addition of a mixture

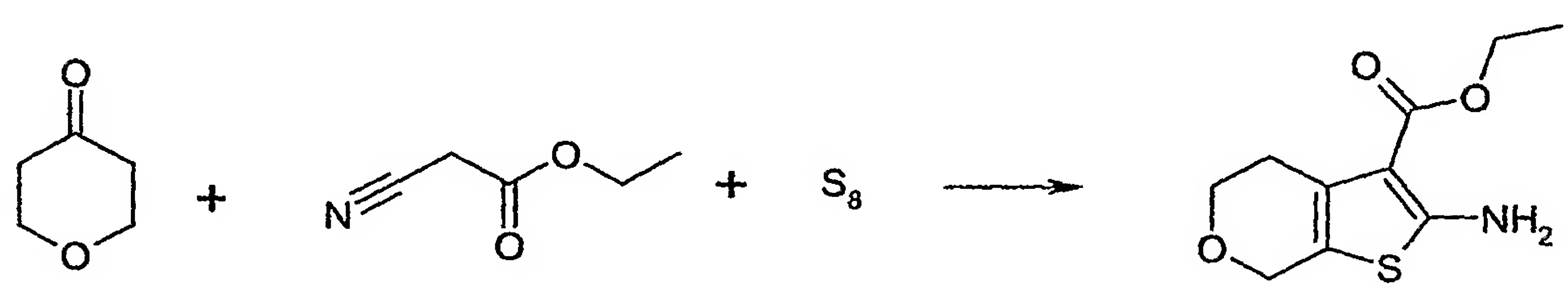
of ammonium acetate and acetic acid in a molar ratio of greater than 1, preferably in the range from 0.5:1 to 0.8 to 1, and preferably at a temperature in the range of 50 to 100 °C, preferably between 70 to 90 °C, preferably under removal of water formed in the reaction, and preferably for a duration of 2 to 4 hours.

Furthermore, in a preferred embodiment of the present invention, in Step I the reaction product of the reaction of compound (B) with the cyano-acetate ester is reacted with the S_8 in a protic solvent, preferably EtOH, S_8 being added at least in aquimolar quantities, preferably in an excess of up to 1,5, more preferably of up to 1,2, in the presence of a amine base, preferably morpholine, at reaction temperature of between 25 to 65 °C, preferably between 40 and 60 °C, and preferably for a duration of 2 to 6 hours.

Examples

Syntheses of compounds

I. Preparation of **Ethyl 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carboxylate**

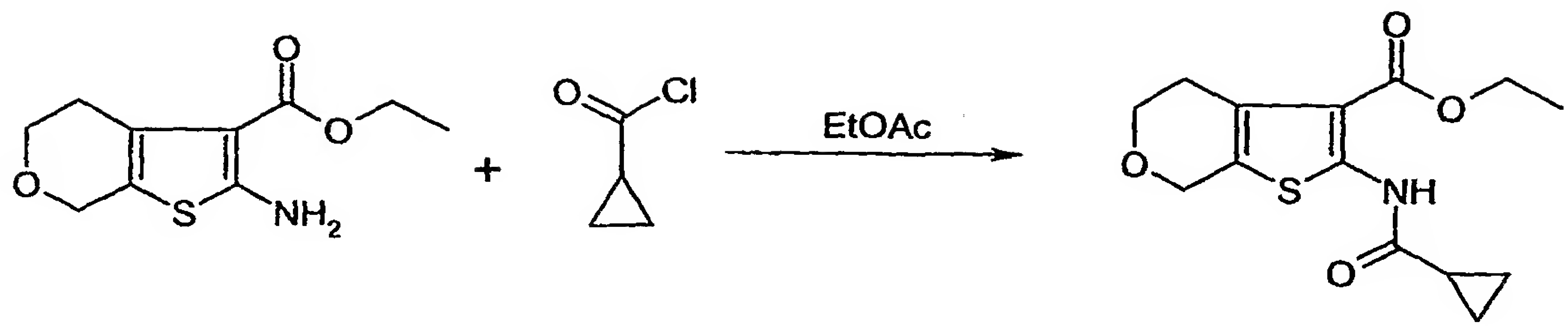


1 mM tetrahydro-pyran-4-one, 106 µL (1 mM) ethyl-cyano-acetate, 0.15 mM ammonium-acetate, and 0.2 mM acetic acid where dissolved in 3 mL benzene and stirred at reflux temperature in a round-bottomed flask equipped with water-remover trap, for 3 hours. The reaction mixture was washed with 2 mL 10% K_2CO_3 solution, dried, and evaporated to dryness. The solid material was dissolved in 1.5 mL EtOH and was stirred with 1.05 mM sulphur and 0.575 mM morpholine at 45-50 °C, for 4 hours. The reaction mixture was evaporated to dryness, washed with n-hexane and isopropylalcohol. This reaction step was developed starting from a procedure described by Gewald, K; Schinke, E; Böttcher, H; *Chem. Ber.* **1966**, *99*, 974.

Yield: 57 %

NMR: 7.28 (s, 2H), 4.43 (s, 2H), 4.16 (q, 2H), 3.79 (t, 2H), 3.67 (t, 2H), 1.25 (t, 3H)

II. Preparation of **2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid ethyl ester**



1 mM cyclopropanecarbonyl chloride was added dropwise to a well stirred, 15 mL ethylacetate solution of 301 mg (1.00 mM) 2-amino-6-phenyl-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester. The reaction mixture was stirred for 3 hours, then diluted to 50 mL, washed two times with water, dried with $MgSO_4$, and evaporated to dryness. The product was washed with n-hexane and isopropanol.
Yield: 42 %

NMR: 11.19 (s, 1H), 4.60 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 2.03 (m, 1H), 1.33 (t, 3H), 0.93 (m, 4H)

Analogous to this method the following compounds were also synthesized:

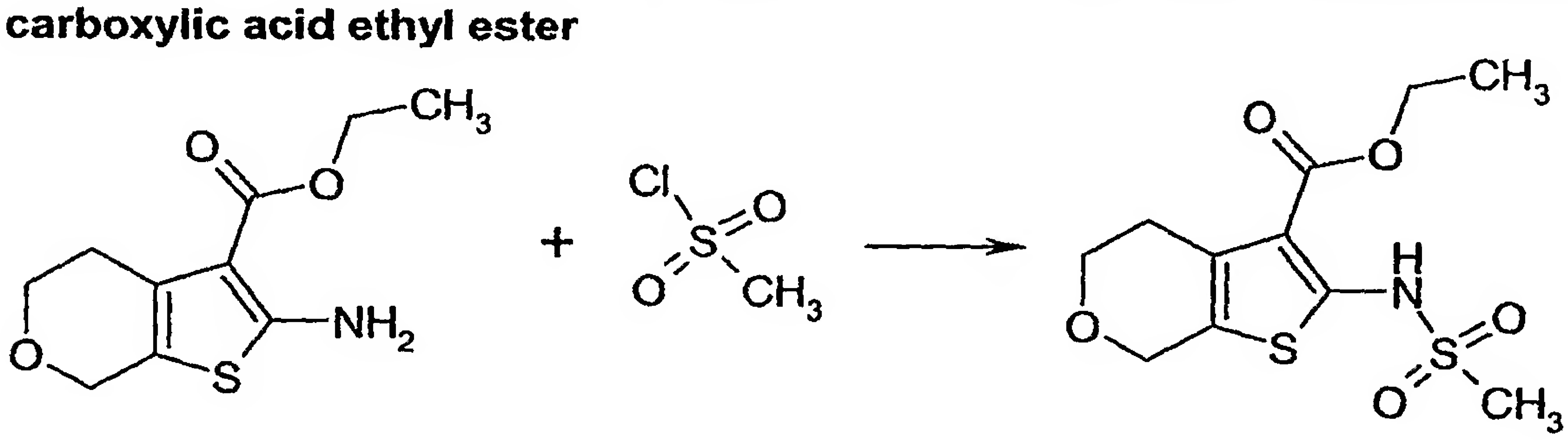
2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 57%), NMR: 11.90 (s, 1H), 8.06 (s, 1H), 7.39 (d, 1H), 6.79 (dd, 1H), 4.66 (s, 2H), 4.35 (q, 2H), 3.86 (t, 2H), 2.82 (t, 2H), 1.35 (t, 3H);
2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 67%), NMR: 11.36 (s, 1H), 4.62 (s, 2H), 4.32 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 2.06 (bs, 2H), 1.90 (s, 8H), 1.72 (s, 6H), 1.33 (t, 3H);
2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 70%), NMR: 11.10 (s, 1H), 4.61 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.90 (d, 2H), 1.69 (m, 3H), 1.43-1.18- (m, 9H);
2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 57%), NMR: 11.15 (s, 1H),4.60 (s, 2H), 4.25 (q, 2H), 3.64 (t, 2H), 2.78 (t, 2H), 1.80 (m, 1H), 1.33 (t, 3H), 1.11 (d, 3H), 0.79 (m, 1H);
2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 76%), NMR: 10.91 (s, 1H), 4.61 (s, 2H), 4.28 (q, 2H),3.83 (t, 2H), 3.44 (m, 1H), 2.78 (bs, 2H), 2.23 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H), 1.31 (t, 3H);

2-Acetylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 85%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);

2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 64%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);

2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 76%), NMR: 11.02 (s, 1H), 6.90 (m, 1H), 6.35 (dd, 1H), 4.63 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 1.92 (s, 3H), 1.89 (s, 3H), 1.32 (t, 3H);
2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 69%), 11.05(s,1H),4.62(s,2H),4.30(q,2H), 3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);
2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (yield 76%), NMR: 11.05(s,1H),4.62(s,2H),4.30(q,2H), 3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);
2-(2-Chloro-acetylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 82%), NMR: 11.64 (s, 1H), 4.64 (s, 2H), 4.61 (s, 2H), 4.32 (q, 2H), 3.85 (t, 2H), 2.80 (t, 2H), 1.32 (t, 3H);
2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 79%), NMR: 11.89 (s, 1H), 7.95 (m, 1H), 7.76 (m, 2H), 4.67 (s, 2H), 4.34 (q, 2H), 3.87 (t, 2H), 2.82 (t, 2H), 1.34 (t, 3H);
2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 80%), NMR: 11.08 (s, 1H), 4.62 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (m, 3H), 1.32 (t, 3H), 1.17 (d, 6H);
2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 77%), NMR: 11.04 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.99 (m, 1H), 2.78 (t, 2H), 1.91 (m, 2H), 1.65 (m, 6H), 1.31 (t, 3H).

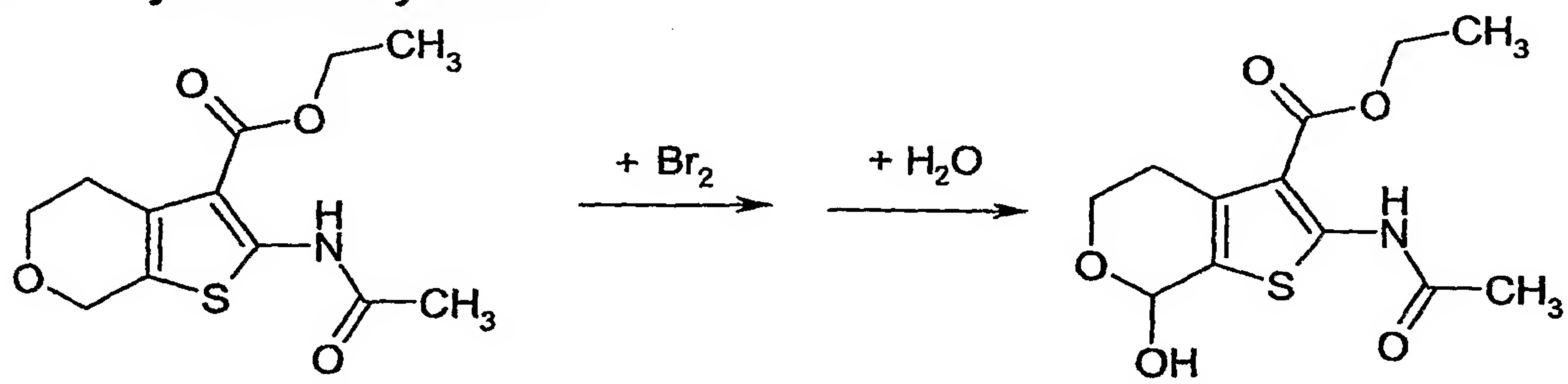
III. Preparation of **2-Methanesulfonylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester**



1 mmol 2-Amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 10 ml benzene and 348 µL (2.5 equivalent) triethylamine, 195 µL (2.5 equiv.) methanesulfonyl chloride was added. The reaction mixture was refluxed for 8 hours. The mixture was extracted with 1x 15mL water, 1x 15 mL $NaHCO_3$, then 1x 15 ml water, 1x 15 mL 1N HCl and saturated NaCl solution. The organic layer was dried

above $MgSO_4$, the solvent was evaporated to vacuo and the residue was crystallized from hexane-isopropanol. (TLC-Eluent: Hexan-Ethylacetate: 2:1)
Yield: 65%, NMR: 11.03 (s, 1H), 4.73 (s, 2H), 4.28 (q, 2H), 3.89 (t, 2H), 3.53 (s, 3H), 2.83 (t, 2H), 1.29 (t, 3H).

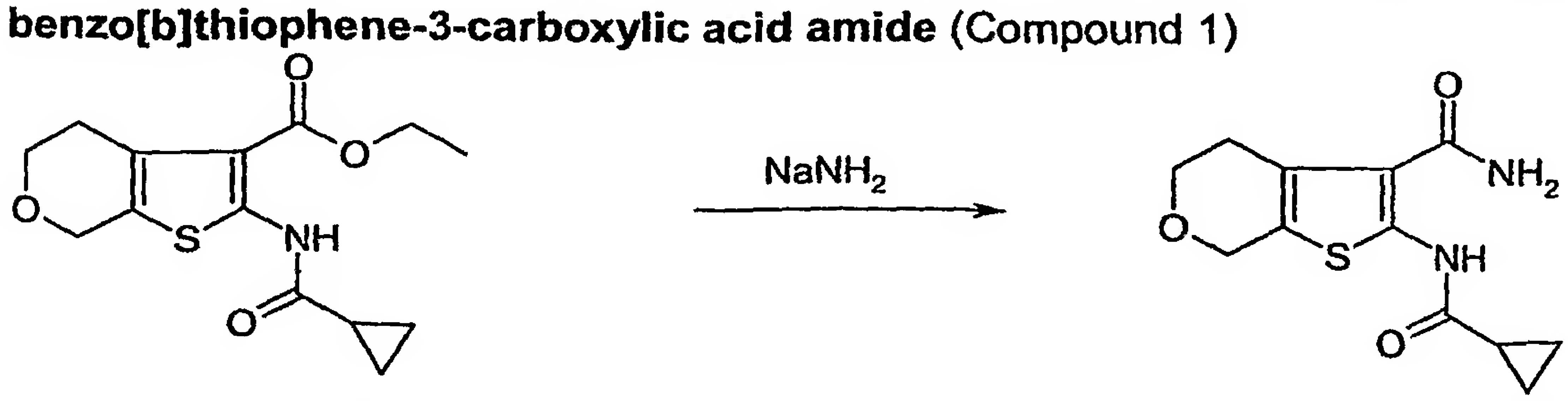
IV. Preparation of **2-Acetamino-7-hydroxy-4,7-dihidro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester**



269 mg (1 mmol) 2-Acetylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 15 mL acetic acid and 82 mg (1 mmol) sodium-acetate was added to the mixture, then heated to 55 ^{0}C. 159 mg bromine in 15 mL acetic acid was added slowly to the mixture. After one hour stirring it was evaporated under reduced pressure and extracted three times with ethyl acetate and 15 mL water. The organic layer was washed with 10 mL $NaHCO_3$ solution and dried with $MgSO_4$. The solution was evaporated under reduced pressure and the product was crystallized from hexane. The product was washed with IPA, and recrystallized with diisopropyl-ether. Yield: 39% NMR: 10.92 (s, 1H), 4.83 (d, 1H), 4.73 (d, 1H), 4.49 (d, 1H), 4.29 (q, 2H), 3.90 (d, 1H), 3.65 (d, 1H), 2.24 (s, 3H), 1.32 (t, 3H).

The compound 2-(Cyclopropanecarbonyl-amino)-7-hydroxy-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was synthesized in a analogous reaction. Yield: 62%, NMR: 11.20 (s,1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H).

V. Preparation of **2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid amide** (Compound 1)

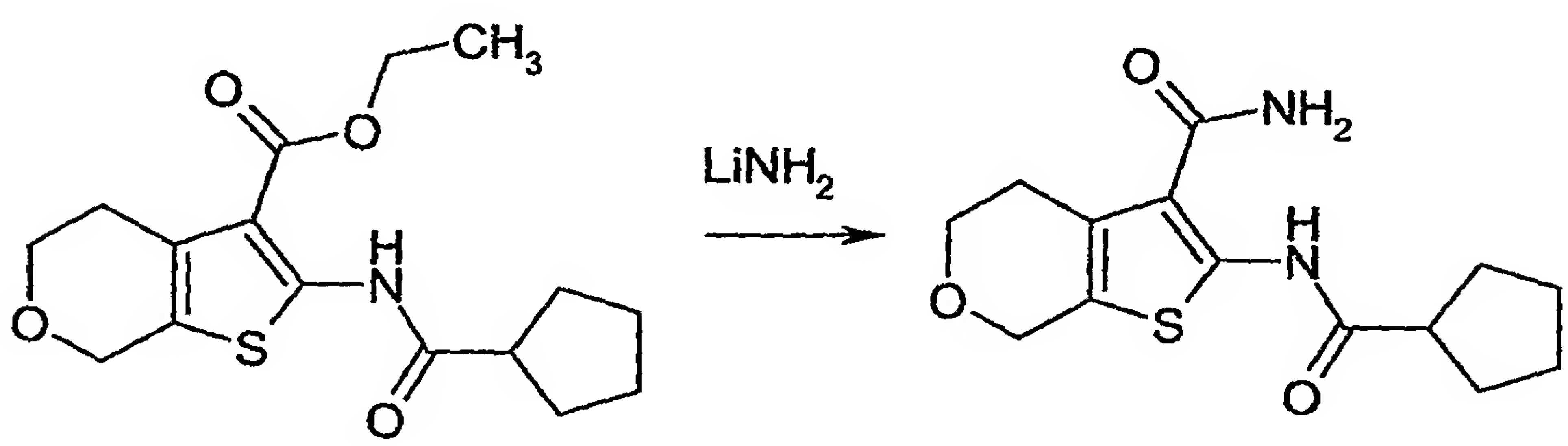


470 mg (12.00 mM) sodium amide was added to the solution of 293 mg (1.00 mM) 2-(cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic

acid ethyl ester in 8 mL abs. tetrahydrofurane. The air-tightly closed reaction mixture was stirred at room temperature for 72 hours.

After the starting material disappeared, the pH of the reaction mixture was set to 5 – 6 with ice cold, 1 N HCl, the precipitated product was filtered off, washed twice with 5 mL n-hexane and dried.
Yield: 89 % white, or off-white crystals; NMR: 11.75 (s, 1H), 4.62 (s, 2H), 3.83 (t, 2h), 2.79 (t, 2H), 1.89 (m, 1H), 0.87 (m, 4H)

VI. Preparation of **2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 2)



1 mmol 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 3 mL abs. THF, then 230 mg (10 equivalent) $LiNH_2$ was added and the mixture was stirred in a stoppered flask at r.t. for 48 hours. The reaction mixture was poured on ice water, the pH of the solution was adjusted to 5 with 5% HCl. The precipitated crystals were filtered out and washed with cold isopropanol. (TLC Eluent: chloroform-MeOH 10:1)
Yield: 79%, NMR: 11.73 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.88 (m, 1H), 2.80 (t, 2H), 1.89 (m, 2H), 1.64 (m, 6H).

The following compounds were also prepared by this method:

2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3), Yield: 67%, NMR: 11.77 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 2.46 (m, 1H), 1.60 (m, 1H), 1.47 (m, 1H), 1.12 (d, 3H), 0.85 (t, 3H);

2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4), Yield: 74%, NMR: 11.63 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 3.33 (m, 1H), 2.81 (t, 2H), 2.20 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H);

2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5), Yield: 73%, NMR: 11.26 (s, 1H), 7.32-7.19

(m, 5H), 4.62 (s, 2H), 4.28 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.55 (m, 1H), 1.43 (m, 1H), 1.30 (t, 3H);

2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6), Yield: 49%, NMR: 11.64 (s, 1H), 7.3 (bd, 2H), 6.83 (m, 1H), 6.23 (dd, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 1.89 (d, 3H);

2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7), Yield 31%, NMR: 11.56 (s, 1H), 7.2 (bs, 2H), 5.93 (s, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 2.16 (s, 3H), 1.90 (s, 3H);

2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8), Yield 32%, NMR: 12.33 (s, 1H), 7.2 (bs, 2H), 4.64 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 1.22 (s, 9H);

2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 9), Yield: 70%; NMR: 13.01 (s, 1H), 7.88 (m, 1H), 7.70 (m, 2H), 7.30 (bs, 2H), 4.69 (s, 2H), 3.86 (t, 2H), 2.86 (t, 2H);

2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10), Yield: 61%, NMR: 11.81 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.81 (t, 2H), 2.67 (m, 1H), 1.14 (d, 6H);

2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 11), Yield: 65%, NMR: 11.20 (s,1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H);

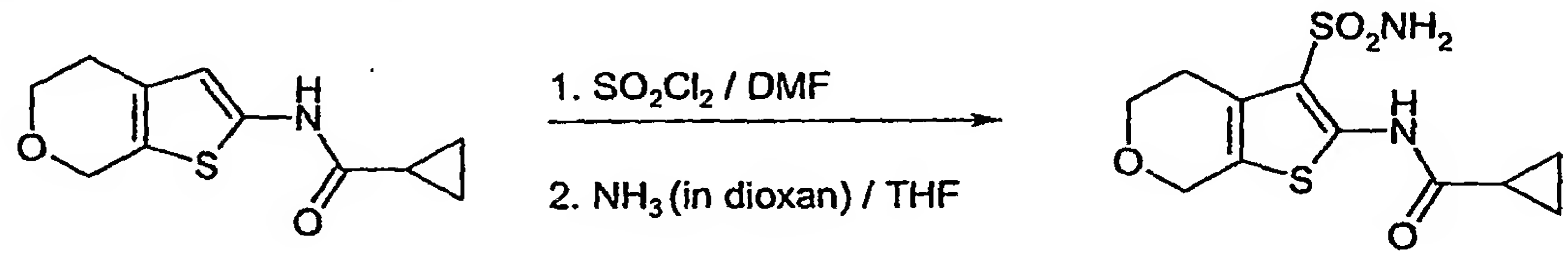
2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 12), Yield: 53%, NMR: 11.71 (s, 1H), 7.5 (bs, 1H), 7.0 (bs, 1H), 4.61 (s, 2H), 3.82 (t, 2H), 2.79 (t, 2H), 1.64 (m, 1H), 1.09 (d, 3H), 0.73 (m, 1H);

2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 13), Yield: 34%, NMR: 12.72 (s, 1H), 8.02 (d, 1H), 7.7 (bs, 1H), 7.31 (d, 1H), 7.2 (bs, 1H), 6.76 (dd, 1H), 4.67 (s, 2H), 3.85 (t, 2H), 2.86 (t, 2H);

2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 14), Yield: 61%, NMR: 12.23 (s, 1H), 7.3 (b, 2H0, 4.63 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 2.03 (s, 3H), 1.86 (s, 5H), 1.70 (s, 5H);

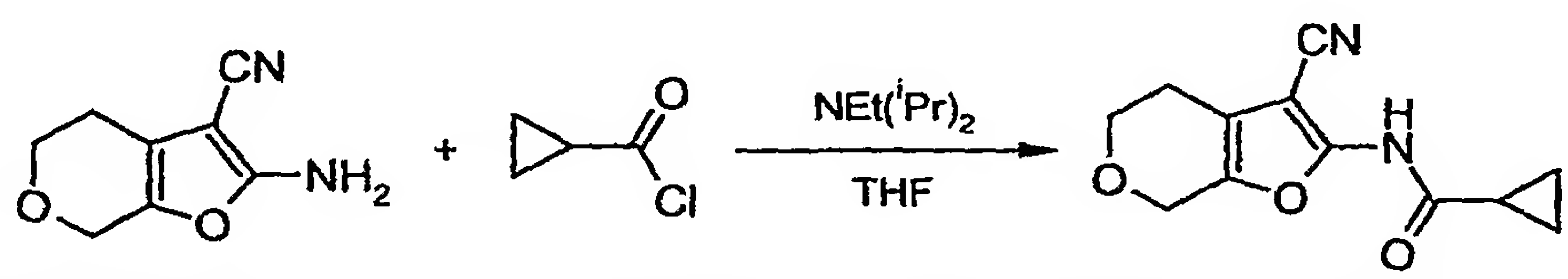
2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 15), Yield: 63%, NMR: 11.79 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.82 (t, 2H), 2.80 (t, 2H), 2.41 (m, 1H), 1.89-1.62 (m, 5H), 1.40-1.17 (m, 5H).

Preparation of **2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide** (Compound 17)



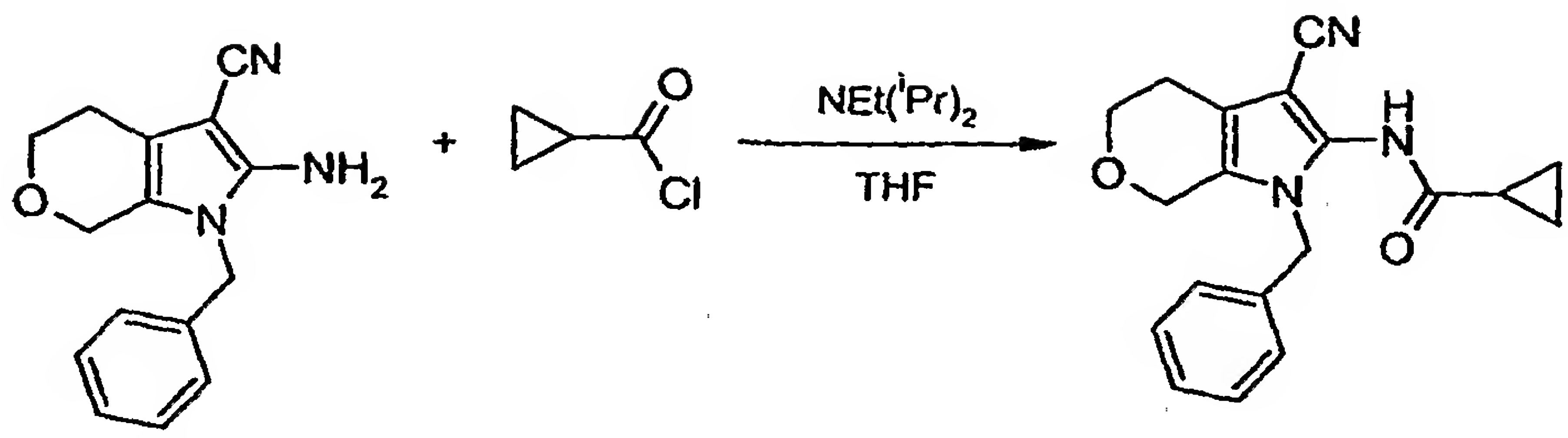
Sulfurylchloride (13 mmol) was added dropwise to DMF (13 mmol) at 0 °C under Argon. The mixture was stirred for 30 min at 0 °C and 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyrane (10 mmol) in 2 ml DCM added. The mixture was stirred for 1 h at r.t., diluted with 2 ml of THF and treated with an excess of NH_3 (2 M solution in dioxane, 10 ml, 20 mmol). The mixture was stirred at room temperature overnight. Evaporation of the solvent and recrystallization afforded the title compound.

Preparation of **2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane**



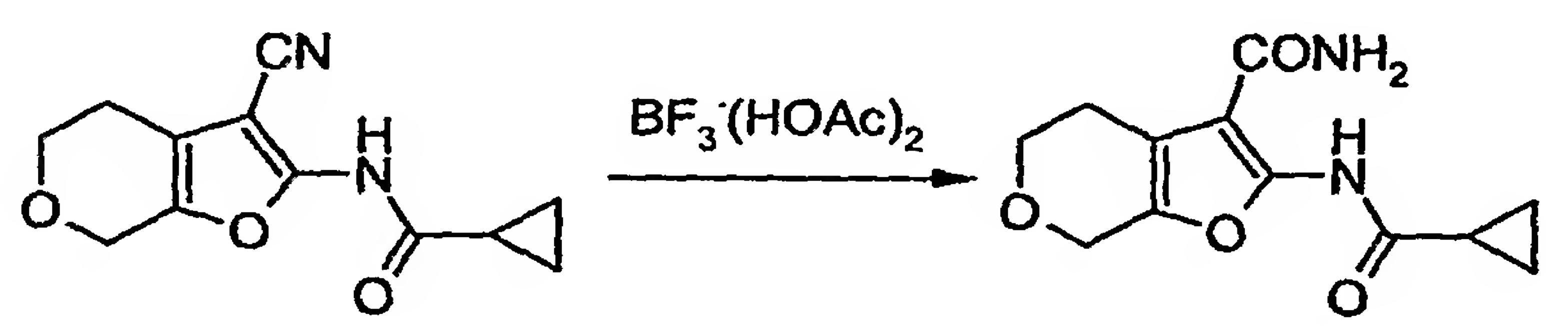
2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (0.66 mmol) and cyclopropylcarbonyl chloride (0.8 mmol) were dissolved in 10 mL of abs. THF. Diisopropylethylamine (0.8 mmol) was added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

Preparation of **1-Benzyl-2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane**



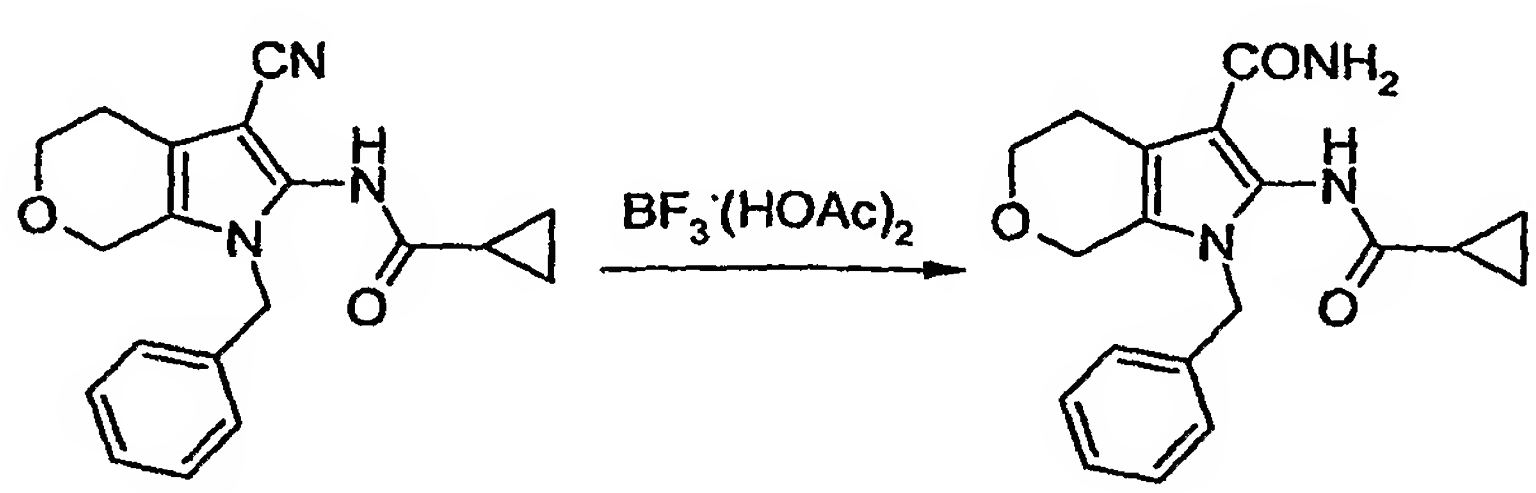
1-Benzyl-2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane (4.1 mmol) and cyclopropylcarbonyl chloride (4.9 mmol) were dissolved in 10 mL of abs. THF. 1.5 mL of diisopropylethylamine were added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

Preparation of **2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5H-furo[2,3-c]pyrane-3-carboxamide**



2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (4.3 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 *N* sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material can be recrystallized from hot ethanol.

Preparation of **1-Benzyl-2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane -3-carboxamide**



1-Benzyl-2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane (4 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 *N* sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material is recrystallized from hot ethanol.

Biochemical methods and experiments

In the following documents, background information is given with regard to the methods, micoorganisms and enzymes used according to the present invention: Peirs et al., *A serine/threonine protein kinase from Mycobacterium tuberculosis*, Eur. J. Biochem., Mar 1, 244(2), 604-612 (1997); Arruda et al., *Cloning of an M. tuberculosis DNA fragment associated with entry and survival inside cells*, Science 261, 1454-1457 (1993); Wieles et al., *Increased intracellular survival of Mycobacterium smegmatis containing the Mycobacterium leprae thioredoxin-thioredoxin reductase gene*, Infect Immun. 65(7), 2537-2541 (1997); Zahrt, *Mycobacterium tuberculosis signal transduction system required for persistent infections*, Proc. Natl. Acad. Sci. 98 (22), 12706-12711 (2001); and Mundayoor et al., *Identification of genes involved in the resistance of mycobacteria to killing by macrophages*, Ann. N. Y. Acad. Sci. 730, 26-36 (1994).

Bacterial strains and culture conditions

Mycobacterium smegmatis was grown in Middlebrook 7H9 medium (supplier: Difco), supplemented with 10% ADC (Difco), 0.05% Tween-80 and 0.5% glycerol. *E. coli* was cultivated in LB- or TB-broth without any additional ingredients. Cloning, mutagenesis and expression of PknG and other mycobacterial kinases was done as described by Koul et. al. (Serine/threonine kinases, PknG and PknF of *Mycobacterium tuberculosis*: characterisation and localisation. Microbiology, 147, 2001).

GST-fusion protein purification

Purification of GST-fusion proteins was done as described previously by Koul et. al. (Serine/threonine kinases, PknG and PknF of *Mycobacterium tuberculosis*: characterisation. and localisation. Microbiology, 147, 2001). *E. coli* BL21 cultures

containing the respective plasmids were grown overnight in TB-broth. After IPTG induction, the suspensions were incubated for another 16 hours at room temperature. The bacteria were harvested by centrifugation, resuspended in 1x PBS and lysed by sonification. After addition of Triton X-100 (1% final concentration) and subsequent clarifying of the lysates the GST-fusion proteins were purified by addition of GST-sepharose following PBS washes. The proteins were eluted with a buffer containing 50 mM glutathion, 20 mM Tris (pH 8.0), 0.1 M NaCl, 0.1 M Triton X-100 and 1 mM DTT. Thereafter, the eluates were dialysed in 20 mM HEPES (pH 7.5) and 30 % glycerol.

Determination of protein kinase activity

The activity of all protein serine threonine kinases from *Mycobacterium tuberculosis* was determined by addition of myelin basic protein as a substrate in an *in vitro* kinase assay. The buffer conditions were as follows: 20 mM HEPES (pH 7.5), 20 mM $MgCl_2$, and 5 mM $MnCl_2$, for all kinases except PknG, PknI, PknJ, and PknL. These protein kinases required lower salt concentrations, namely 1 mM $MgCl_2$, and 1 mM $MnCl_2$. The optimum ATP concentration for each kinase was determined by titration of ATP in a range between 0.0033 μM and 100 μM. The inhibitor studies were performed with ATP concentrations similar to the Michaelis constant (K_m) for ATP. We further analysed the role of PknG in pathogenesis of mycobacteria in cellular infection model.

Infection of macrophage cells with recombinant *Mycobacterium smegmatis*

Mycobacterium smegmatis, electroporated with either vector alone or mycobacterial expression vector containing PknG (wild type) or PknG-K181M (Mutant), was cultured for 2 days in Middlebrook 7H9 medium containing 0.05% Tween-80 and 0.5% glycerol. Bacteria were pelleted at 1500 x g for 3 minutes by centrifugation and resuspended by vigorous agitating (Vortex) in Dulbecco's modified Eagle's medium (DMEM, GIBCO-BRL, Gaithersburg, USA)) containing 5 % fetal bovine serum (FBS) for infecting murine macrophage cell line RAW (American Type Culture Collection No. 91B-71). This yielded a bacterial supernatant consisting mostly of single mycobacterial cells as observed by acid fast staining. Under the assumption that an optical density (O.D.) of 0.1 at 650 nm equals to 10^8 CFU/ml (see in this respect Wei et al., *"Identification of a Mycobacterium tuberculosis Gene that enhances survival of M. smegmatis in Macrophages"*, J. Bacteriol. 182, 377-384 (2000)), the O.D. of *Mycobacterium smegmatis* cell suspension was measured and diluted to 5 x 10^6

CFU/ml in DMEM containing 5 % FBS. Viable counts were performed on Middlebrook 7H10 medium.

The RAW cell line was maintained in DMEM medium supplemented with 10 % FBS. The survival assay for recombinant *Mycobacterium smegmatis* was performed as described by Wei et al., cited above. RAW cells were plated in a 24 well tissue culture plate (4 x 10^5 cells/well) and incubated overnight in 5 % CO_2 at 37°C. The inoculum (1 ml) containing 5 x 10^6 recombinant *Mycobacterium smegmatis* was added to achieve muliplicities of infection (moi) of 10. The plate was incubated for 2 hours at 37°C in 5 % CO_2. The infected monolayers were washed twice with warm DMEM and treated with 2 % FCS containing 200 µg of amikacin/ml for 1 hour at 37°C to kill extracellular *M. smegmatis*. The cells were again washed twice with warm DMEM and further incubated in DMEM containing 20 µg of amikacin. This time point was considered 0 hours of infection. The 24 hours infected monolayer was incubated with 20 µg of amikacin/ml to prevent extracellular growth of any bacteria released by premature lysis of infected RAW cells. Cells were washed twice with warm DMEM before lysis was effected by addition of a 0.1 % SDS solution and vigorously pipeting several times to ensure lysis of cells and release of surviving bacteria. The lysates were diluted in 7H9 broth and plated onto 7H10 agar plates and CFU were counted after incubation at 37°C for 4 to 5 days.

Validation of mycobacterial kinase as a mycobacterial virulence gene

Mycobacterium smegmatis was electroporated either with wildtype or mutant kinase (which exerts no kinase activity) or vector control. Mouse macrophage cell line (RAW) was infected with the various recombinant *M. smegmatis* expressing either pknG wild type or PknG K/M mutant or vector alone. After infection, the cells were lysed at different time points and the amount of intracellular bacteria was analysed. As can be seen from Fig. 1, after one hour postinfection the amount of bacteria recovered from macrophages infected with *M. smegmatis* expressing PknG wild type or K/M mutant or vector alone was the same. This shows that the recombinant M. smegmatis strains were internalised with equal efficiency. However, after 24 hour postinfection the amount of *M. smegmatis* transformed with the vector control or the mutant kinase was substantially decreased within macrophages. This shows an efficient clearance or degradation of the the *M.smegmatis* expressing vector alone or PknG K/M mutant by the lysosomal degradation pathway with in the macrophages.

But in contrast, after 24 hrs an approximately tenfold increased amount of M.smegmatis survived within the cells expressing wildtype PknG compared to the mutant. This clearly demonstrates that the kinase activity of PknG increases the intracellular survival of *M. smegmatis* within macrophages and as such makes PknG an important virulence factor of mycobacteria. Consequently, the kinase is a promising target for recognising, monitoring, and controlling therapy of various diseases.

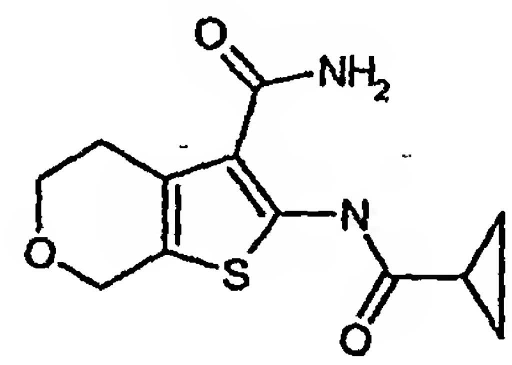
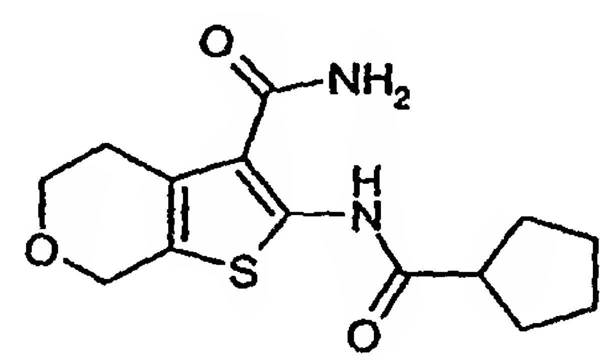
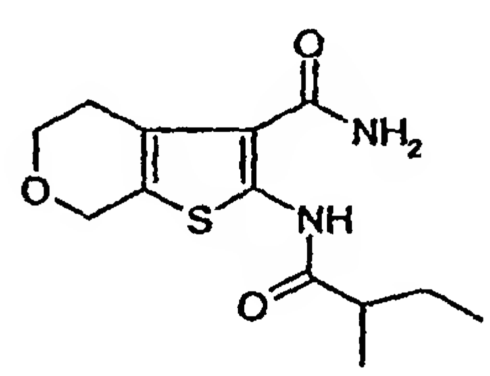
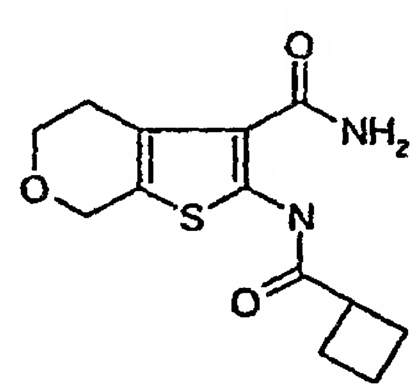
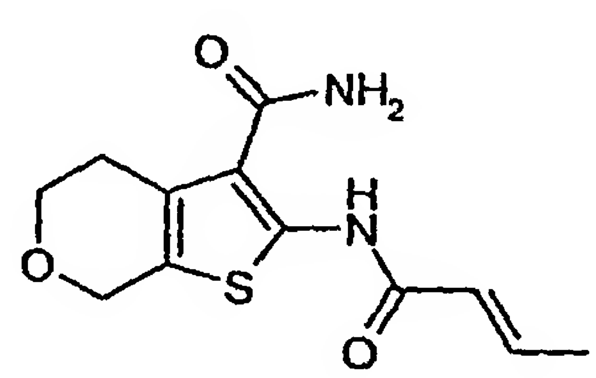
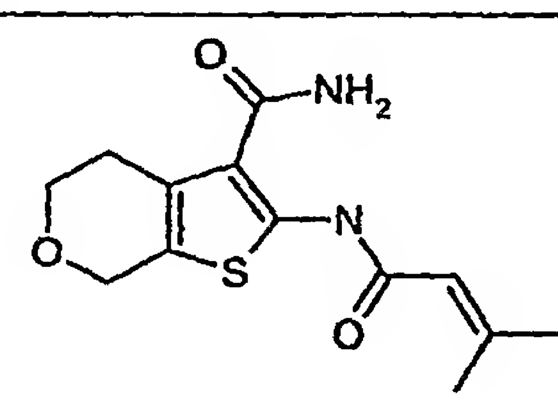
Screening for inhibitors of PknG

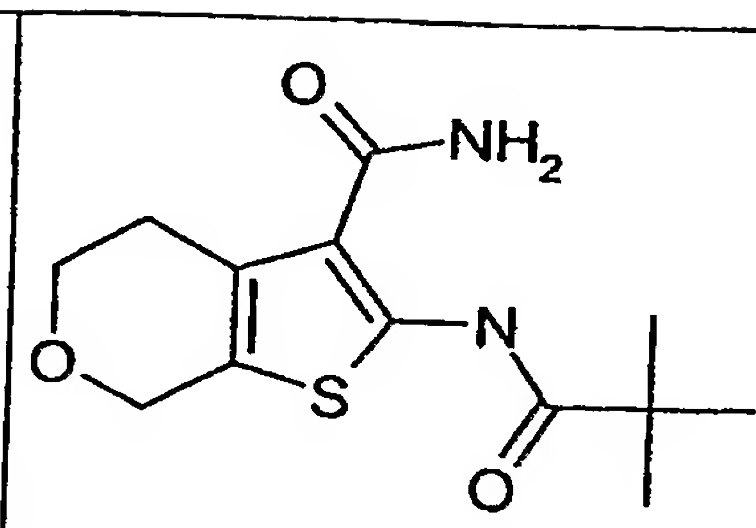
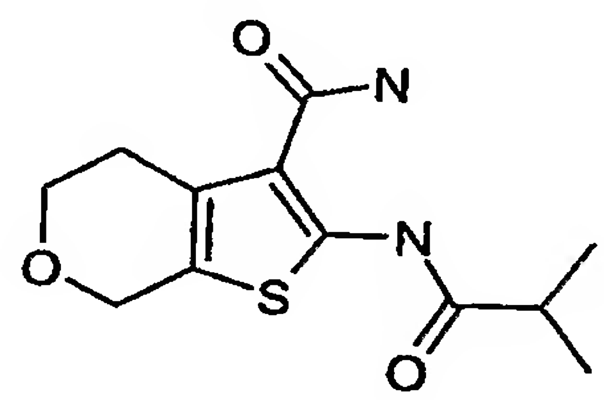
A search was conducted for specific molecules inhibiting the target kinase (PknG) of *Mycobacterium tuberculosis*. In a kinase platform a suitable substrate was identified and an *in vitro* assay was adapted to high throughput screening. Subsequently, a library comprising 55.000 compounds using the established *in vitro* kinase assay was screened. Table I shows the half-maximal inhibition constant (IC_{50}) values of the compounds 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 1), 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2), 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3), 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4), 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6), 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7), 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8), 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10), for inhibiting mycobacterial PknG .

As is evident from Table I, compound 1 is the most effective compound of those tested in inhibiting the activity of protein serine/threonine kinase G of *M. tuberculosis*, compound 1 having an IC_{50} value of only 490 nm. With compounds 2, 3, 4, 6, 7, 8, and 10 satisfactory results were also obtained, the compounds having IC_{50}-values, between about 2μm and up to about 70 μm.

Table I

Inhibitory effect on mycobacterial protein kinase G (PknG)of selected compounds according the present invention

Compound No.	Structure	Inhibition of PknG (IC_50, [µM])
Compound 1: 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0.49**
Compound 2: 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**3,42**
Compound 3: 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**69,2**
Compound 4: 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**2.17**
Compound 6: 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**2,26**
Compound 7: 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**2,64**

Compound 8: 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**57,3**
Compound 10: 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**20,43**

<u>Secretion of PknG outside the bacterial cell</u>

In the following it is demonstrated that PknG is secreted outside the cell into the culture supernatant by mycobacterial cells.

1) PknG and ESAT (a secretary protein that acts as a positive control) are cloned in BamH1 site of pYUB 2401. This vector contains the promoter for hsp60. A in-frame fusion with the start of hsp60 and phoA at the C-terminus by cloning into the BamH1 site. The vector is kanamycin resistant. After cloning PknG and ESAT in pYUB2401 they were electroporated in *M. smegmatis* and the colonies were grown on LB plates with 40µg of 5-bromo–4-chloro-3-indoylphosphate (BCIP) and with 20 µg of kanamycin used for screening.

PhoA fusion proteins that are exported beyond cytoplasm are enzymatic ally active and capable of hydrolysing the BCIP, the chromogenic substrate of PhoA to produce the blue colonies.

2) *M. smegmatis* strains containing either
   1) ESAT-PhoA
   2) PknG-PhoA or
   3) PhoA alone

were grown in 7H9 medium with kanamycin to saturation for 5-6 days and then diluted to the final optical density (O.D.) of 0.005 at 600 nm.

3) These cultures were then grown for 40 hours at 37 ^{0}C. The OD_{600} of each strain was measured at the start of the experiment.

4) A 0.5 ml portion of the cell culture was pelleted and resuspended in equal volume 1 M Tris (pH. 8.0).

5) Then 0.1 ml of cells was added to1.0 ml of 2 mM p–nitrophenyl phosphate plus sodium salt in 1 M Tris (pH 8.0).

6) The reaction was incubated in dark at 37 ^{0}C until a yellow reaction product was formed.

7) Next , 0.1 ml of 1 M K_2HPO_4 was added to terminate the reaction.

8) The bacteria were pelleted and the OD_{420} of 1.0 ml of the supernatant was measured.

9) Alkaline phosphatase activity units were determined by the following formula:

$$\frac{1000 \times OD_{420}}{OD_{600} \times 0.1 \text{ ml volume of cells}}$$

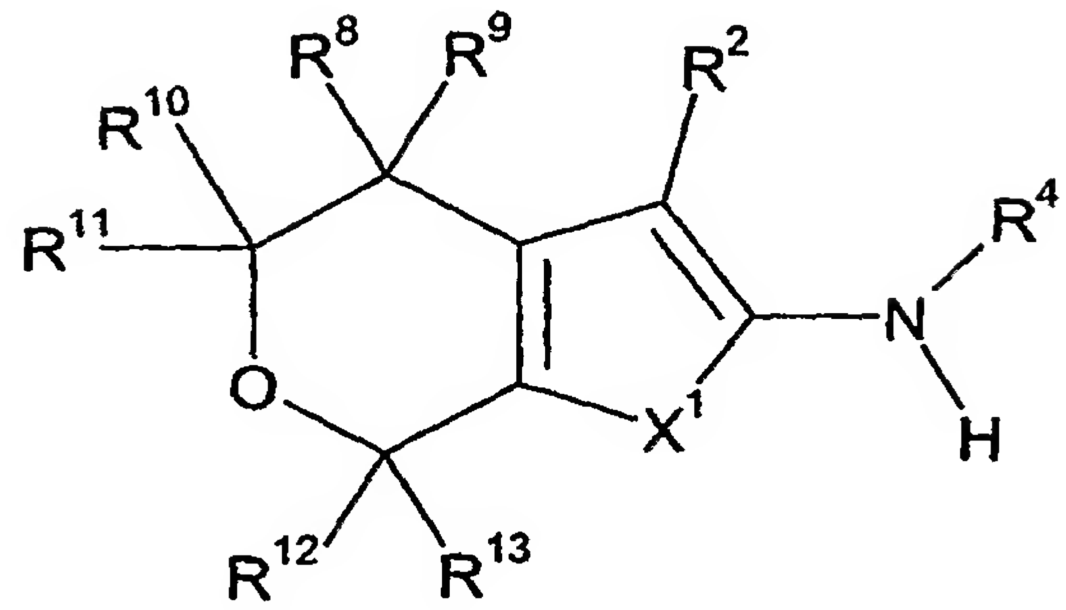
The negative control will be the *M. smegmatis* cells alone and PhoA transfected *M. smegmatis*.

The above method is described in Braunstein M, Griffin TJ IV, Kriakov JI, Friedman ST, Grindley ND, Jacobs WR Jr., *„Mycobacterium tuberculosis proteins using a Tn552'phoA in vitro transposition system"*, J Bacteriol. 2000 May;182(10):2732-40.

The result of the above-mentioned experiment shows that PknG is a secretory protein that is secreted outside the mycobacterial cells. Fig. 3 shows the alkaline phosphatase secretions assay for PknG for different PhoA fusion constructs. The secreted PknG can phosphorylate host cell proteins that might be critical in survival of mycobacterium in host cells.

Claims

1. Compounds having the general formula (I)



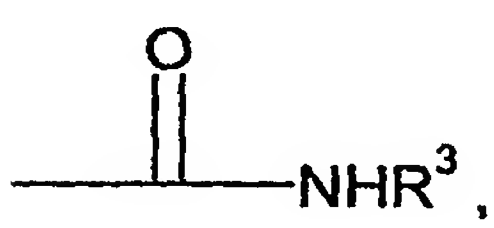
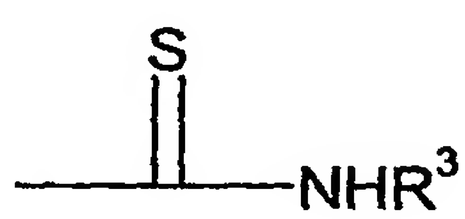
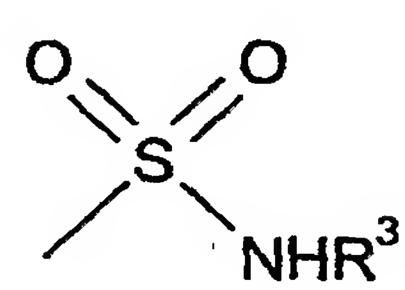
(I)

wherein

X^1 is selected from S, O, NR^1,

and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is selected from

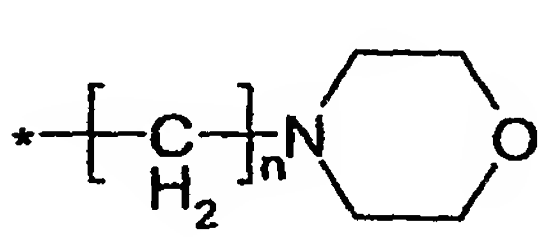
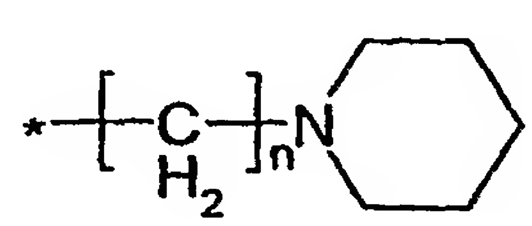
, , and  ,

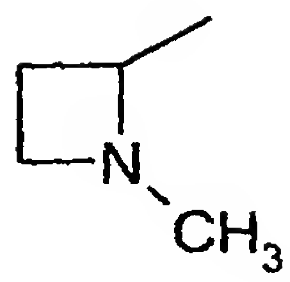
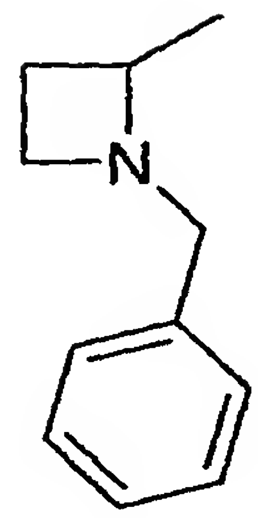
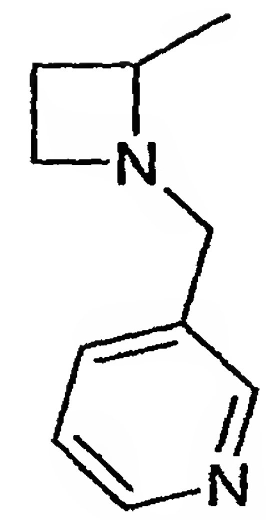
wherein R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_6-alkyl,

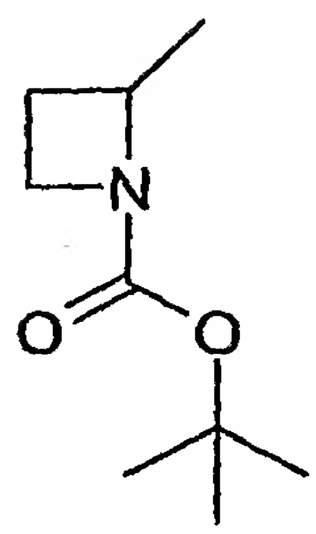
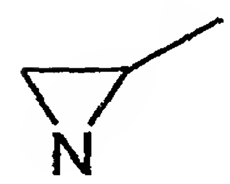
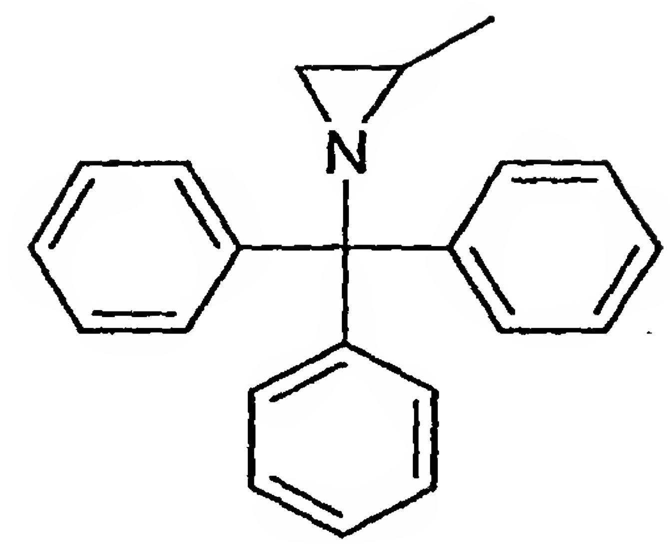
R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,

 ,  ,

,
,
,

,
,


or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and

R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

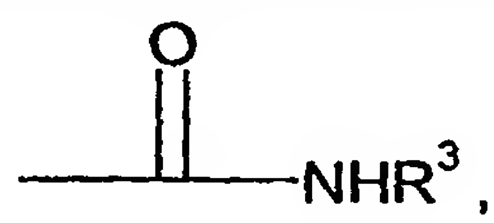
and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

2. The compound according to claim 1, wherein X^1 is S.

3. The compound according to claim 1, wherein X^1 is NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

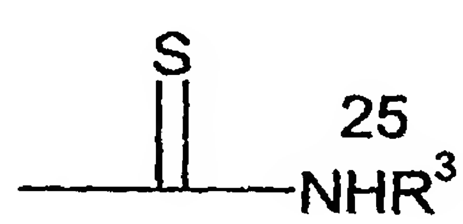
4. The compound according to claim 1, wherein X^1 is O.

5. The compound according to any one of claims 1 to 4,

wherein R^2 is  ,

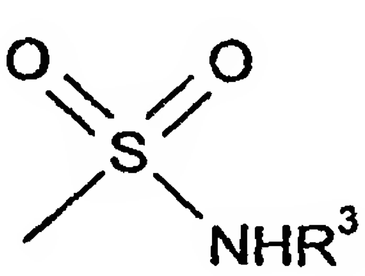
and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

6. The compound according to any one of claims 1 to 4,

wherein R^2 is  ,

and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

7. The compound according to any one of claims 1 to 4,

wherein R^2 is 

and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

8 The compound according to any one of claims 1 to 7, wherein R^3 is selected from the group consisting of H, $-CH_2-CH_2-OH$, $-CH_2-CH_2-NH_2$, $-CH_2-CH_2-SH$, $-CH_2-CH(OH)-CH_3$, $-CH_2-CH(SH)-CH_3$, or $-CH_2-CH(NH_2)-CH_3$.

9. The compound according to any one of claims 1 to 8, wherein R^4 is -C(=X^2)R^5 and X^2 is O or S.

10. The compound according to claim 9, wherein X^2 is O

11. The compound according to any one of the preceding claims, wherein R^4 = SO_2-R^5.

12. The compound according to any one of claims 1 to 11 wherein R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, adamantyl, or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

13. The compound according to any one of claims 1 to 12, wherein R_5 is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, *cis*- or *trans*-prop-1-enyl, but-1-enyl, adamantyl, 3,4-difluorophenyl or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^7 preferably is phenyl or 3,4-difluorophenyl.

14. The compound according to claim 13, wherein R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

15. The compound according to claim 13 or 14, wherein R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkoxy, or OH.

14. The compound according to any one of claims 1 to 13, wherein R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1-C_6-alkyl.

15. The compound according to any one of claims 1 to 14, wherein R^8 and R^9 are both H.

16. The compound according to any one of claims 1 to 15, wherein R^{10}, R^{11}, R^{12}, and R^{13} are independently selected from H and substituted or unsubstituted C_1-C_6-alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl or tert.-butyl.

17. The compound according to any one of claims 1 to 16, wherein R^{10} and R^{11} are methyl and R^{12} and R^{13} are H or wherein R^{10}, R^{11}, R^{12}, and R^{13} are H or wherein R^{10}, R^{11}, R^{12}, and R^{13} are methyl or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.

18. The compound according to any one of claims 1 to 15, wherein R^{10} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

19. The compound according to any one of claims 1 to 15, wherein R^{12} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

20. The compound according to any one of claims 1 to 19, wherein R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl or benzyl.

21. The compound according to any one of claims 1 to 20, wherein R_{14} and R_{15} are independently selected from methyl, ethyl and n-propyl. iso-propyl or allyl, and preferably are methyl.

22. The compound according to anyone of claims 1 to 21, wherein the compound is selected from the group consisting of:

2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 1),

2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2),

2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3),

2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4),

2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5),

2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6),

2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7),

2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8),

2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 9),

2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10),

2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 11),

2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 12),

2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 13),

2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 14),

2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 15),

5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 16), and

2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide (Compound 17).

23. A compound according to claims 1 to 22 for use as a pharmaceutically active agent.

24. A compound according to claim 23 wherein the compound is used for treating diseases and/or infections, particularly virally and/or bacterially induced diseases or infections.

25. The compound according to claim 24, wherein the bacterially induced disease is one caused by a bacterium of the genus legionella.

26. The compound according to claim 25, wherein the disease is legionnaires' disease.

27. The compound according to claim 24, wherein the bacterially induced disease or infection is caused by a mycobacterium.

28. The compound according to claim 25, wherein the mycobacterium is *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

29. The compound according to claim 27 or 28, wherein the disease is tuberculosis, leprosy or mycobacterially induced meningitis.

30. A compound according to claims 1 to 22 for use as an inhibitor for a protein kinase.

31. The compound according to claim 30, wherein the protein kinase is secreted from a cell to an environment of the cell.

32. The compound according to claims 30 or 31, wherein the protein kinase is a mycobacterial kinase.

33. The compound according to claim 32, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

34. The compound according to claim 33, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).

35. Use of at least one compound according to independent claims 1 to 22 as a pharmaceutically active agent.

36. Use according to claim 35 for treating diseases and/or infections, particularly virally and/or bacterial induced diseases or infections.

37. Use according to claim 36, wherein the bacterially induced disease or infection is caused by a mycobacterium or a bacteria of the genus legionnella.

38. Use according to claim 37, wherein the mycobacterium is *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

39. Use claim 37 or 38, wherein the disease is tuberculosis, leprosy or mycobacterially induced meningitis, or in the case that the bacteria is of the genus legionella is legionaires' disease.

40. Use according to claim 35 for use as an inhibitor for a protein kinase.

41. Use according to claim 40, wherein the protein kinase is secreted from a cell to an environment of the cell.

42. Use according to claims 40 or 41, wherein the protein kinase is a mycobacterial kinase.

43. Use according to claim 42, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

44. A compound according to claim 43, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).

45. Use of at least one compound according to claims 1 to 23 for the preparation of a pharmaceutical composition for the treatment of virally and/or bacterial induced diseases or infections, preferably legionnaires' disease, tuberculosis, leprosy and mycobacterially induced meningitis.

46. Use of at least one compound according to claims 1 to 22 for the preparation of a pharmaceutical composition.

47. Use according to claim 46, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of diseases and infections, particularly virally or bacterially induced diseases or infections.

48. Use according to claim 47, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of diseases caused by a bacterium of the genus legionella.

49. Use according to claim 47, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of legionnaires' disease.

50. Use according to claim 47, wherein the bacterially induced disease or infection is induced by a mycobacterium.

51. Use according to claim 50, wherein the mycobacterium is *Mycobacterium tuberculosis or Mycobacterium leprae*.

52. Use according to any one of claims 50 or 51, wherein the disease is tuberculosis, leprosy, or mycobacterially induced meningitis.

53. Use according to any one of claims 50 to 52, wherein the compound inhibits a protein kinase, particularly the protein serine/threonine kinase G (PknG) of *Mycobacterium tuberculosis* and/or *Mycobacterium leprae*.

54. Use according to claim 53, wherein the protein serine/threonine kinase G (PknG) is secreted from the interior of the *Mycobacterium tuberculosis* or *Mycobacterium leprae* to the environment of the respective *Mycobacterium*.

55. Method for preventing or treating a disease or infection in a mammal, including a human, wherein the method comprises the administration of a pharmaceutically effective amount of at least one of the compounds according to claims 1 to 22 to the mammal.

56. The method according to claim 55, wherein the disease or infection a virally or bacterially induced diseases or infections.

57. The method according to claim 56, wherein the bacterially induced disease or infection is induced by a mycobacterium or by a bacteria of the genus legionella.

58. The method according to claim 57, wherein the mycobacterium is *Mycobacterium tuberculosis or Mycobacterium leprae*.

59. The method according to any one of claims 57 to 59 , wherein the disease is tuberculosis, leprosy and/or mycobacterially induced meningitis, or in the case that the bacteria is of the genus legionella is legionniares disease.

60. Method for amidation of an carboxylic acid ester to give the corresponding primary carboxylic acid amide,

comprising the step of reacting an carboxylic acid ester with an alkali metal amide in the presence of a polar solvent, which is essentially inert against the alkali metal amide, wherein the molar ratio of carboxylic acid ester to alkali metal amide lies in the range of 1:1 to 1: 15.

61. The method according to claim 60, wherein the molar ratio of carboxylic acid ester to alkali metal amide lies in the range of 1:5 to 1:13 and preferably in the range of 1:9 to 1: 13.

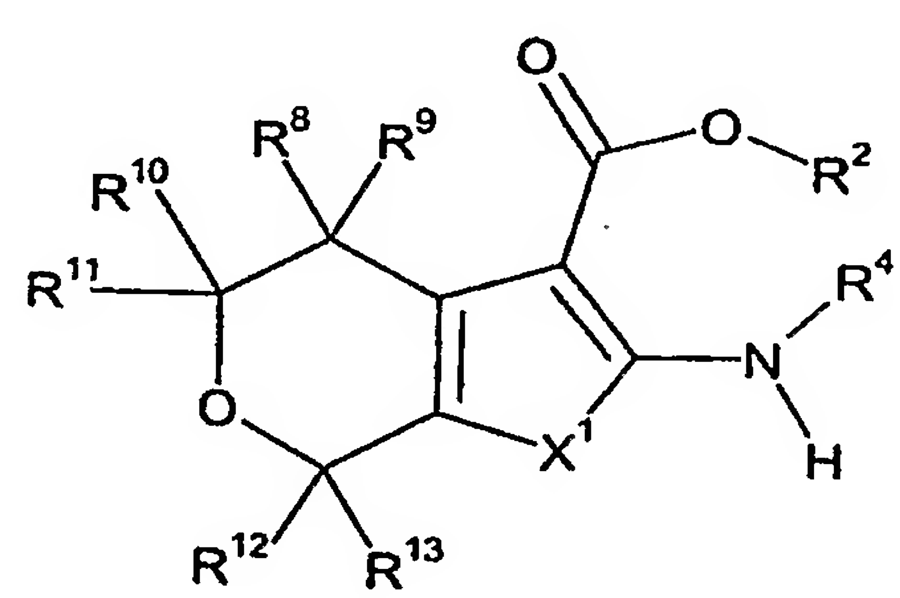
62. The method according to claim 61 or 62 wherein the alkali metal amide is $LiNH_2$ or $NaNH_2$, and preferably $LiNH_2$.

63. The method according to any one of claims 60 to 62 wherein the solvent in absolute ether or absolute tetrahydrofurane, preferably tetrahydrofurane, and the reaction is preferably carried out under the exclusion of moisture.

64. The method according to anyone of claims 1 to 4 wherein the reaction is carried out at a temperature of 15°C to 35°C, preferably at 25°C.

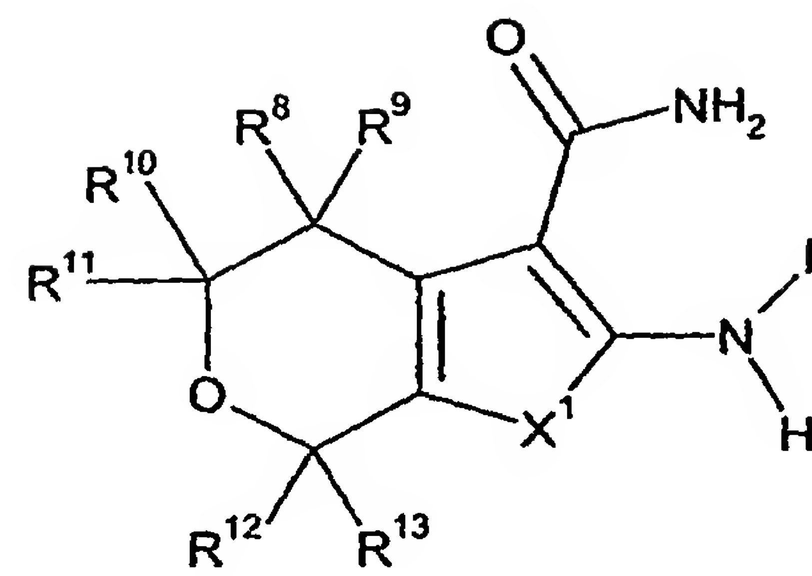
65. The method according to anyone of claims 60 to 64, wherein the reaction duration lies in the range of from 40 to 80 hours, preferably from 45 to 75 hours.

66. The method according to any one of claims 60 to 65, wherein the carboxylic acid ester is a compound according to the following general formula (D):



(D)

which is amidated to give the primary carboxylic acid amide according to formula (E),



(E)

wherein in formulas (D) and (E)

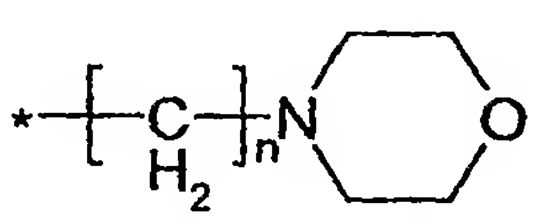
X^1 is selected from S, O, or NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is linear or branched C_1-C_6 alkyl or aryl and preferably is methyl, ethyl, phenyl or benzyl,

R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,



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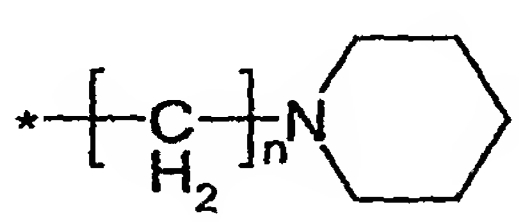

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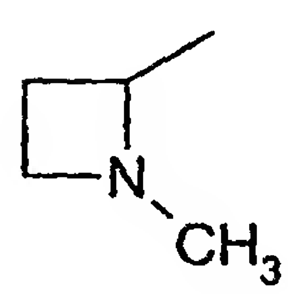

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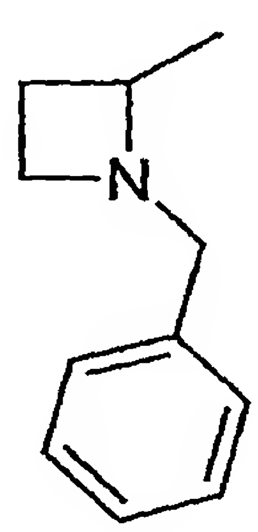

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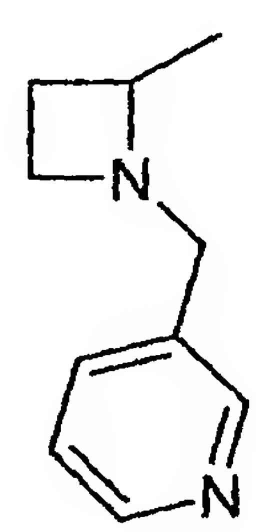

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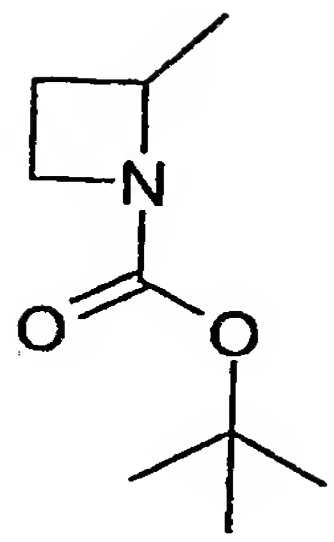
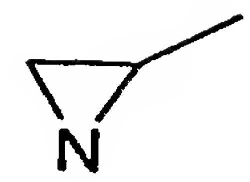
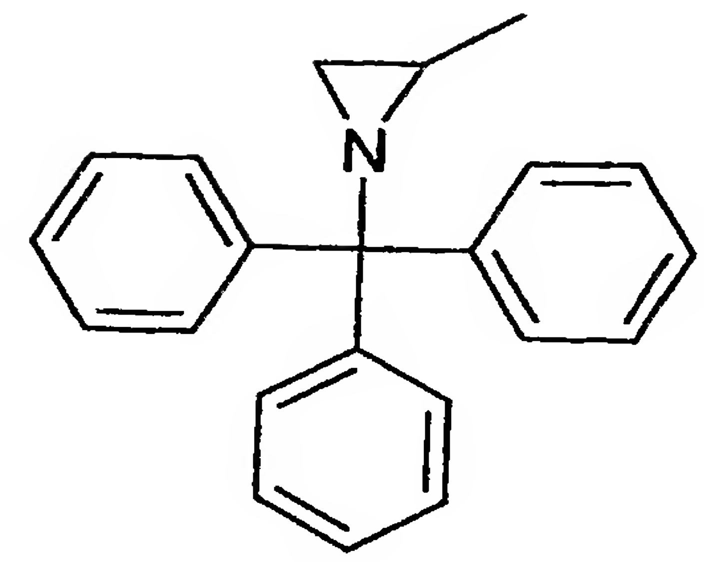
,
,
,

or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and

R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

67. The method according to claim 7, wherein in general formulas (D) and (E)

X^1 is S

R^2 is methyl or ethyl,

R^4 is $-C(=O)R^5$ and R^5 is selected from methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl,

trans-but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH,

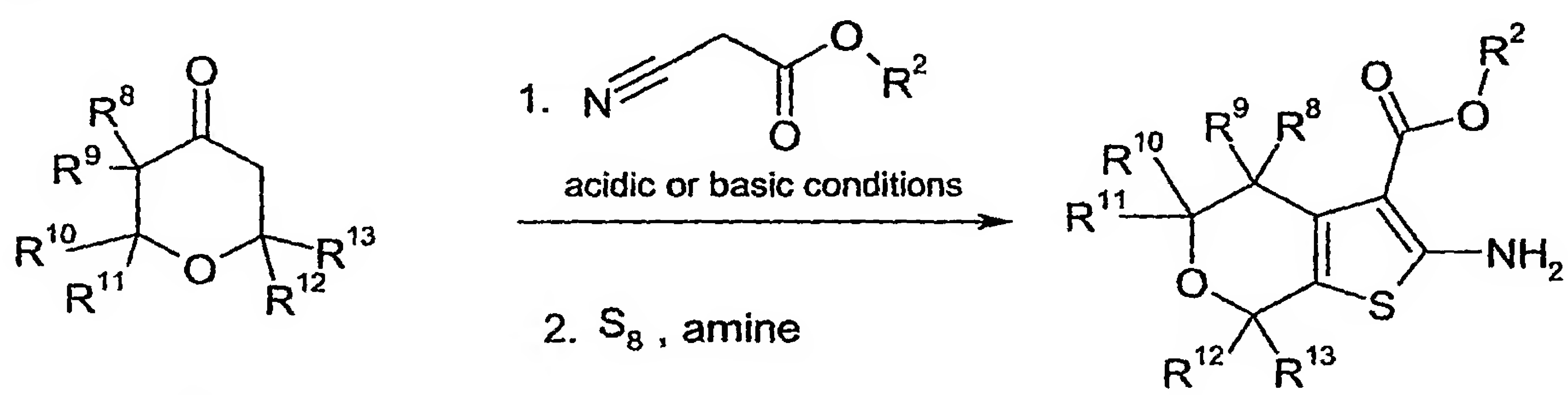
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl,

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

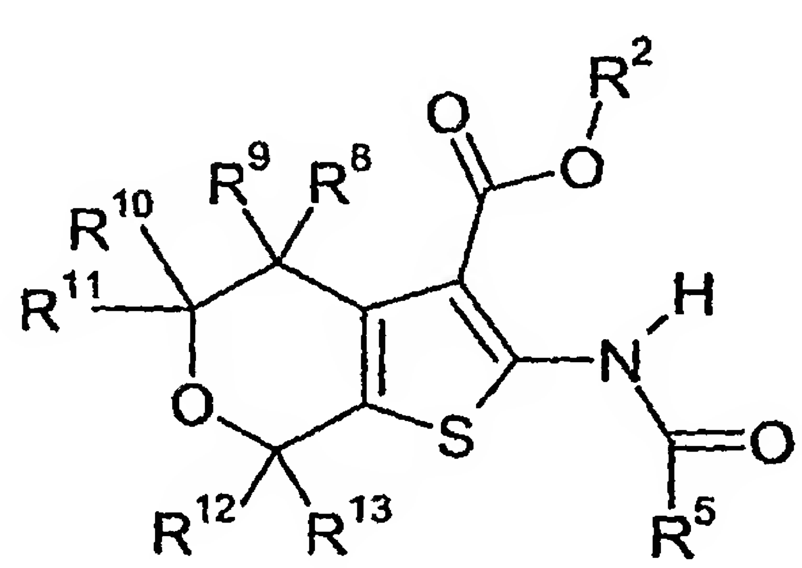
68. The method according to claim 67 in which the compound according to the general formula (E) is obtained by the following reaction sequence:

Step I:



(B) (C)

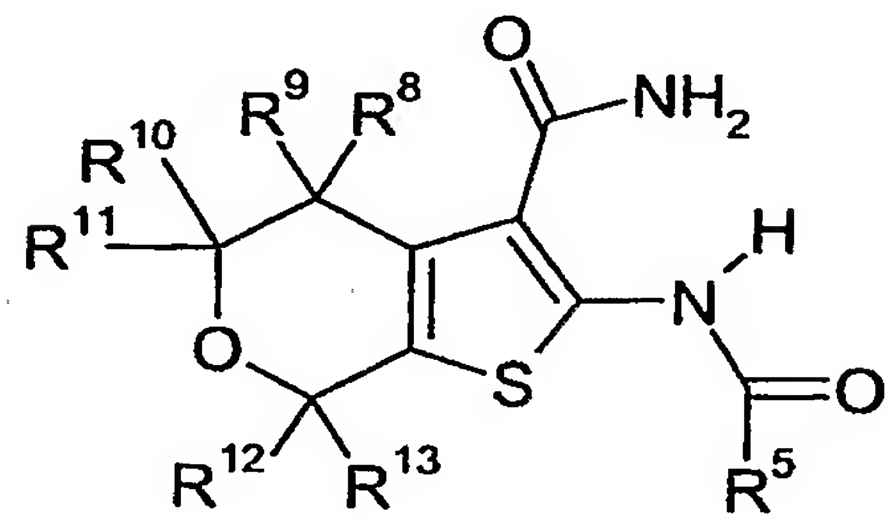
Step II: acylation of the $-NH_2$ group in 2-position in compound C with $R^5C(=O)LG$, wherein LG represents a suitable leaving group, preferably a halogen such as F, Cl, Br or I, most preferably Cl, to give compound (D):



(D)

and,

- Step III: Amidation of as outlined in any one of claims 60 to 65, to give compound (E):



(E)

69. The method according to claim 68, wherein in Step I the reaction of compound (B) with the cyano-acetate ester is carried out in a nonpolar solvent, preferably benzene, with the addition of a mixture of ammonium acetate and acetic acid in a molar ratio of greater than 1, preferably in the range from 0.5:1 to 0.8 to 1, and preferably at a temperature in the range of 50 to 100 °C, preferably between 70 to 90 °C, preferably under removal of water formed in the reaction, and preferably for a duration of 2 to 4 hours.

70. The method according to any one of claims 68 or 69, wherein in Step I the reaction product of the reaction of compound (B) with the cyano-acetate ester is reacted with the S_8 in a protic solvent, preferably EtOH, S_8 being added at least in aquimolar quantities, preferably in an excess of up to 1,5, more preferably of up to 1,2, in the presence of a amine base, preferably morpholine, at reaction temperature of between 25 to 65 °C, preferably between 40 and 60 °C, and preferably for a duration of 2 to 6 hours.

Summary

Described are 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues and pharmaceutically acceptable salts thereof, the use of these derivatives for the prophylaxis and/or treatment of mycobacteria-induced infections and opportunistic infections, as well as compositions containing at least one 7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues derivative and/or pharmaceutically acceptable salts thereof.

PCT/EP2004/010161